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THE INFLUENCE OF DIETARY IODIDE ON THE PLASMA IODIDE LEVELS OF BROOK TROUT, *SALVELINUS FONTINALIS* (MITCHILL)

by

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ABSTRACT

Variable and high plasma iodide levels are found in brook trout, Salvelinus fontinalis (Mitchill). The influence of dietary iodide on the plasma iodide levels of brook trout held at 13° C was examined by force-feeding the fish gelatin capsules containing supplementary iodide. Following capsule administration of radioiodide 58 to 69% of the dose occurred in the trout body suggesting efficient iodide absorption from the gut.

Trout force-fed for 10 days an amount of iodide corresponding to that contained in an iodide-rich food (Ewos) had significantly increased plasma iodide levels. The mean maximum level attained (1860 μg I/100 ml) was similar to that of trout fed Ewos food for 11 months (1740 μg I/100 ml) suggesting that trout quickly reach an upper plasma iodide limit. Starvation for 11 and 37 days significantly decreased the plasma iodide levels.

The natural diet of brook trout contained less iodide than Ewos food but more than four other foods examined. A significant dietary iodide source therefore exists in the natural habitat.

Neither the total plasma protein concentration nor the plasma protein affinity for iodide, as determined by somewhat extreme but standardized conditions of dialysis, were correlated with the plasma iodide levels and were not factors influencing the variable plasma iodide levels. The only factor other than dietary iodide which contributed to the plasma iodide variability was the state of female sexual maturity.

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Brook Trout were supplied by the Province of Manitoba Trout Hatchery, West Hawk Lake, Manitoba.

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INTRODUCTION

Plasma iodide levels recorded for fresh-water teleosts range from 0.5 μg I/100 ml in a European cyprinid, bream, *Abramis brama*, to 2244 μg I/100 ml in a clupeiform, shad, *Alosa alosa*, during upstream migration (Leloup, 1970). Levels of approximately 20 to 800 μg I/100 ml have been found in the plasma of the eastern brook trout, *Salvelinus fontinalis* (Dorey, 1970). Variable plasma iodide levels were also apparent in brook trout held under uniform conditions (Higgs and Eales, 1971). Higgs and Eales (1971) showed that plasma iodide levels influenced radioiodide parameters used to study thyroid function and noted that high and variable levels could result in misleading interpretation of the radioiodide indices. It is therefore of value to know the factors which influence the plasma iodide levels.

Internal factors which might contribute to plasma iodide variation include differences in (i) thyroid uptake of plasma iodide, (ii) rate of iodide excretion (Leloup and Fontaine, 1960), (iii) thyroid hormone deiodination and iodide absorption from the gut due possibly to starvation (Eales, unpublished) and (iv) binding of iodide to plasma proteins (Leloup and Fontaine, 1960).

External factors influencing plasma iodide levels are the iodide concentration of the water (McNabb, 1963; Hunn and Reineke, 1964; Leloup, 1970) and of the food (LaRoche _et_ _al_., 1965). Hunn and Reineke (1964) found that rainbow trout, _Salmo_ _gairdneri_, could extract iodide from water supplemented with 10 µg I/100 ml and that these trout had tissue iodide levels similar to sea-run California steelheads and greater than trout in low iodide content water. Leloup (1970) showed that iodide was actively transported across the gills and that the rate of iodide uptake was inhibited by hypophysectomy and reinstated after injection of thyroxine or triiodothyronine.

In demonstrating the effectiveness of radiothyroidectomy, LaRoche _et_ _al_. (1965) compared plasma iodide levels in (i) rainbow trout given the radioiodide treatment, (ii) trout fed a low iodide content food (0.1 µg I/g dry diet) for 9 months and (iii) trout fed a high iodide content food (6 µg I/g dry diet) for the same period. The fish fed the iodide-rich diet had significantly greater plasma iodide levels than did either of the other two groups. This suggests that iodide is obtained from food. However, McNabb (1963) compared the iodide contribution to rainbow trout from the food and the water and concluded that water iodide was the more important source. However, he fed the trout a hatchery food (2.66 and 2.88 µg I/g dry diet) and supplemented the water with 30 µg I/l, an amount exceeding fresh water levels (Table I).

The mechanism and control of iodide uptake from water have been demonstrated (Leloup, 1970). However, iodide levels recorded for different fresh water areas range from 0.087 µg I/l (McNabb, 1963) to 5 µg I/l (Robertson and Chaney, 1953) and Hickman (1962) observed little seasonal change in a single fresh-water lake. It is therefore unlikely that active iodide uptake which was found to be controlled by thyroid hormones (Leloup, 1970) would account for the high and variable plasma iodide levels (20 to 800 µg I/100 ml; Dorey, 1970) found in brook trout. The food is an external iodide source and within a single fresh-water area the food is subject to variable iodide levels.

Thus the main objective of this study has been to examine the influence of dietary iodide on the plasma iodide levels of brook trout. To this end, (i) the efficiency of dietary iodide uptake, (ii) the influence of ingested iodide for short and long periods of time, (iii) the possible contribution of iodide from the natural diet and (iv) possible explanations for variable plasma iodide levels have been considered.

TABLE I. Iodide Levels Recorded for Fresh Water

Author	Water Source	µg I/l
Robertson and Chaney, 1953	L. Michigan and L. Superior (6-12 mi. off shore)	5
	Black River (a spawning stream on s. shore of L. Michigan)	≤ 1
Hickman, 1962	Lac Ste. Anne (large eutrophic lake near Edmonton)	0.297
McNabb, 1963	Univ. of Alberta (dechlorinated water)	0.087
Hunn and Reineke, 1964	Mich. State University	≤ 1
LaRoche, Johnson and Woodall, 1965	Indoor hatchery	0.25 to 0.40

LITERATURE REVIEW

A. Iodide Source

Fish require iodide for thyroid hormone production and must ultimately obtain this iodide from an environmental source. The two possible sources are the water and the food.

Hunn and Reineke (1964) found that rainbow trout extracted iodide from water supplemented with 10 μg I/l and Hickman (1962) observed plasma iodide levels in whitefish, _Coregonus clupeaformis,_ up to 3000 times greater than the iodide content of the ambient water (Table I). The scales and mucus make the fish integument highly impermeable to water (and iodide) (Fromm, 1968) and ingestion of iodide from fresh water is negligible (McNabb, 1963; Leloup, 1970). Leloup (1970) showed that water iodide was actively transported across the gills. This transport was reduced after hypophysectomy or treatment with anti-thyroid drugs and was reinstated following injection of thyroxine or triiodothyronine.

In fresh water, the rate of iodide uptake to the blood from water for the European eel, _Anguilla anguilla_, carp, _Cyprinus Carpio_, and rainbow trout ranged from 0.101 to 0.471 μg I/100 g fish/24 hr (Leloup, 1970).

Rainbow trout held in fresh water and fed an iodide supplemented food (6 μg I/g dry wt of food) for 9 months had significantly greater plasma iodide levels than trout fed the food with no additional iodide (LaRoche *et al*, 1965). The food is therefore an environmental source of iodide. However, the mechanism and control of iodide uptake from the gut have not been examined in teleosts.

McNabb (1963) studied the relative contributions of iodide from the water and the food for rainbow trout and concluded that water iodide predominated. However, he fed the fish a hatchery food (2.66 and 2.88 μg I/g dry wt of food) and supplemented the water with 30 μg I/l, an amount exceeding that found in fresh water (Table I).

B. Uptake of Iodide by the Tissues

1. Blood

Plasma iodide levels are influenced by the iodide content of the food (LaRoche *et al*, 1965), the iodide content of the water (Leloup, 1970), water temperature (Leloup and Fontaine, 1960), age of the fish (Leloup, 1970), state of smoltification (Leloup, 1970) and season (Hickman, 1962).

Leloup (1970) recorded plasma iodide levels for 8 Clupeiformes, each in different life stages, 6 Cypriniformes, and 1 Anguilliforme. The values ranged from 0.5 μg I/100 ml for *A. brama*, to 591 (range 134 to 2244) μg I/100 ml for *A. alosa* during upstream migration. In general, the Clupeiformes have the highest plasma iodide levels.

Leloup and Fontaine (1960) found iodide bound to plasma proteins in several diadromous Clupeiformes. Iodide binding was confirmed by Huang and Hickman (1968) who observed significant binding in four Clupeiformes but not in other Orders examined. Huang and Hickman (1968) and Leloup (1970) identified the binding protein as a mammalian-like albumin.

Leloup and Fontaine (1960) advocate that iodide binding is a means of iodide storage for use during migration and is a possible explanation for the high plasma iodide levels found in diadromous clupeiform fish.

2. Thyroid

Environmental iodide is necessary for normal thyroid function. Insufficient iodide can cause thyroid hyperplasia. Marine and Lenhart (1910) alleviated thyroid hyperplasia in brook trout by adding iodine (Lugol's solution) to the water and Marine (1914) eliminated this condition and prevented its reoccurrence by feeding brook trout the high iodide content marine butter fish, Stromateus triacenthus, instead of hog's liver and heart muscle. Woodall and LaRoche (1964) observed slight thryoid hyperplasia in chinook salmon, Oncorhynchus tshawytscha, fingerlings fed food containing 0.1 μg I/g dry wt but not in fingerlings fed food with 0.6 μg I/g dry wt Wild rainbow trout from Lake Michigan which were ripe or spent had low plasma iodide levels and thyroid hyperplasia but this was not so for trout in an area of higher iodide content food and water (Robertson and Chaney, 1953).

The total iodide content of normal thyroid tissue and the relative amounts of the various constituents for several teleosts are shown in Table II. The total iodide values range from 0.1 to 17.87 μg I/100 g body wt and this does not appear to be a size-dependent variation. The iodotyrosines account for approximately 50% of the total iodide and where measured, diiodotyrosine (DIT) is more abundant than monoiodotyrosine (MIT).

3. Other Tissues

Maturing ovaries of teleosts concentrate large amounts of iodide (Robertson and Chaney, 1953; Leloup and Fontaine, 1960; Tarrant, 1971). Lindsey *et al* (1966) suggest that this may serve as an iodide store for the developing embryo.

Tissue to medium ^{131}I ratios (*in vitro*; Nandi and Bern's medium) for the digestive tract, kidney, head kidney, liver, spleen, heart, skeletal muscle and brain of rainbow trout were less than 1.0 but greater than values obtained from boiled tissues (Maqsood *et al*, 1961). *In vitro* tissue to plasma (T/P) ^{131}I ratios for several of the same tissues were also less than 1.0 in the Atlantic salmon, *Salmo salar* L., and rainbow trout (Leloup, 1970) and the channel catfish *Ictalurus punctatus* Refinesque (Tarrant, 1971). Leloup (1970) and Tarrant (1971) examined skin of these fish and

TABLE II. The Iodide Content of the Thyroid of Several Teleosts.

Species	wt. (g)	Thyroid Iodide (µg for a 100 g fish)	Constituents containing the iodide	Author
Salmo gairdneri	453 - 540	0.1 - 0.74 (2.4 - 4.0 µg in thyroid region)		Robertson and Chaney, 1953
Salmo gairdneri	40 - 80		10 days after ^{125}I injection and 22 hr after thyroid digestion: II = 15% T_4 = 9-12% T_3 = 3% MIT & DIT = 40-45% Unknown = 30-35%	Jacoby and Hickman, 1966
Salmo gairdneri	39.2 - 48.9 ($\bar{x}$ = 44.6)	4.71 (2.1 µg in thyroid region)	Origin = 6.5% II = 12% T_4 = 19% DIT = 34% MIT = 14%	LaRoche, Johnson and Woodall, 1965
Oncorhynchus tshawytscha	24.6 - 56.8 ($\bar{x}$ = 39.8)	7.79 (3.1 µg in thyroid region)	Origin = 8% II = 24% T_4 = 6% DIT = 37% MIT = 16%	LaRoche, Johnson and Woodall, 1965

TABLE II (Continued)

Species	wt. (g)	Thyroid Iodide (μg for a 100 g fish)	Constituents containing the iodide	Author
Coregonus clupeaformis		17.87	PBI=16.37 μg/100 g fish BEI=0.6 μg/100 g fish II = 0.5 μg/100 g fish	Hickman, 1962
Anguilla anguilla		2		Leloup and Fontaine, 1960
Searus quacamacia	560 - 900		PBI=1.2-6.4 μg in thyroid region	Matty, 1960

found a fractional tissue to plasma ^{131}I ratio. Wiggs (1967) observed extensive iodide uptake by the burbot, *Lota lota*, skin and commented (from work by Vinogradov, 1953) that Gadiformes were the only teleosts so far examined with high skin iodide concentrations. Leloup (1970) suggested that iodide uptake by extrathyroidal and extraovarian tissues is inversely related to the capacity of plasma proteins to bind iodide.

C. The Natural Diet of Brook Trout

The natural diet of brook trout depends on fish size and age (Ricker, 1930), substrate type and food availability (Ricker, 1930) and season (Needham, 1930; Benson, 1953; Monat, 1965), but there is only slight variability in the food items present. Aquatic insects, in particular Ephemeroptera, Trichoptera, and Diptera larvae constitute approximately 50% or more of the stomach volume (Richer, 1930; Needham, 1930; Benson, 1953). Terrestrial insects are found to a lesser extent. The relative abundance of each Order varies but the major ones found are Coleoptera, Diptera, and Odonata (Ricker, 1930; Needham, 1930; Benson, 1953). Crayfish and fish become a more predominante part of the diet of brook trout greater than 6 to 7 inches in length (Ricker, 1930; Monat, 1965; Lackay, 1969).

MATERIALS AND METHODS

A. Fish Maintenance

The brook trout were obtained from the Province of Manitoba Trout Hatchery, West Hawk Lake, Manitoba. At the hatchery they were held outdoors in cement troughs with a continuous water supply from West Hawk Lake. They were fed Glencoe, Minneapolis pellets until the summer of 1971 when their diet was changed to Ewos trout pellets (Astra Chemicals Ltd.).

In the laboratory fish were held in fiberglass aquaria (150 or 600 gal) to which aereated and dechlorinated water at 13° C was continuously supplied. Stock fish were fed Ewos trout pellets (size #4) until September 1971 and Victor Fox crumbled pellets (Victor Fox Foods Ltd.) thereafter.

Experimental fish were held in 45-gal aquaria in a controlled environmental room at 15 ± 1° C with a 12-L:12-D light regime.

B. Source and Collection of Aquatic Invertebrates

The aquatic invertebrates were collected at several times during May 1972 from the Rat River. The area sampled was above a dam approximately 40 miles south-east of Winnipeg. A dip net was dragged along the bottom and the acquired invertebrates placed in a container of river water until analysed.

C. Feeding of Experimental Fish

Three different methods were used to feed the fish. The first simulated hatchery conditions, a known weight of pellets was offered to the fish. No record was kept of the amount ingested. This method was employed in the long-term feeding experiment. The second method was to force-feed the fish an experimental solution contained within gelatin capsules. The third technique was to voluntarily feed the trout such that a known amount was injested. The latter two methods which were used most frequently are described below in detail.

1. Force-feeding of gelatin capsules to trout

To assure that each trout ingested a known amount of iodide (or thyroid hormone), the fish were force-fed gelatin capsules containing the required solution. Potassium iodide (KI) was the source of stable (non-radioactive) iodide. Carrier-free $Na^{125}I$ was purchased from Atomic Energy of Canada Ltd., Commercial Products, Ottawa. A stock solution of KI in double-distilled water was prepared before the feeding experiment. A fresh radioiodide solution was made every three days and mixed with a known quantity of KI solution (experimental solution) and with double-distilled water (control solution).

Monoido-L-tyrosine (MIT) and diiodo-L-tyrosine (DIT) (Nutritional Biochemicals Corp., Cleveland, Ohio) and 3,5,3'-triiodo-L-thyronine (T_3) and L-thyroxine (T_4) (Sigma Chemicals Co., St. Louis, Miss.) were dissolved in 1 N NaOH, diluted with double-distilled water and neutralized with 1 N HCl. The control solution was the solvent for these organic compounds. The fish which were force-fed stable T_3 and T_4 also received 0.05 μCi ^{125}I-L-thyroxine (T_4^*), specific activity approximately 40 μCi/μg, purchased from Amersham/Searle. The T_4^* solution was prepared every three days during the feeding experiment and was mixed with a quantity of the stable T_3 and T_4. The T_4^* was 94.8% pure, the remainder being inorganic iodide. In all the feeding experiments each fish received 0.1 ml of solution daily.

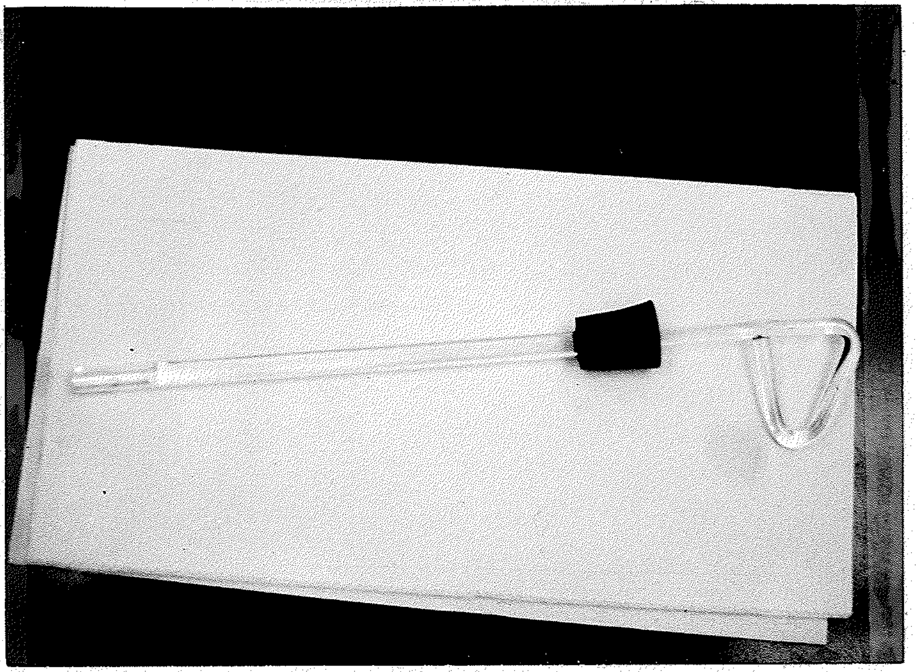
Gelatin capsules (Parke Davis, size 4) were used to administer the solutions. The required number of capsules was placed in a series of holes drilled in a 1"x4"x15" board. The trout were anesthesized in 0.005% tricaine methane sulphonate (MS222) which was adjusted to pH 7. While six to eight trout were in the anesthetic, 0.1 ml of the solution was pipetted into a corresponding number of capsules. The number of capsules filled at one time was important because the liquid caused the capsule to shrivel after 2 to 3 minutes.

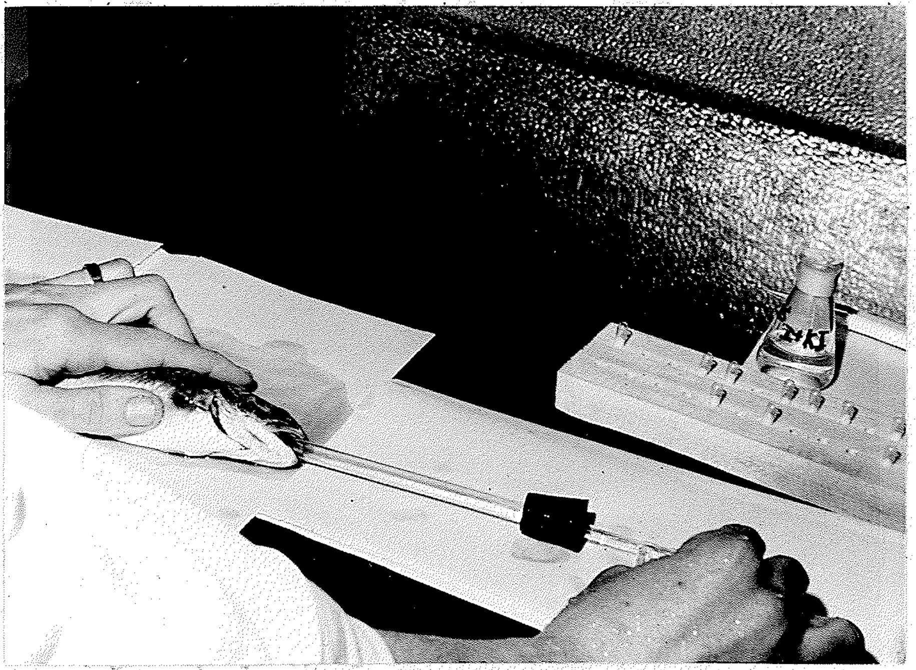
The capsules were force-fed to the fish using an apparatus (Fig. 1) consisting of a glass tube (I.D. 4 mm)

FIGURE 1. Apparatus used to force-feed the trout.

top: Glass tube and rod with capsule in the left end.

bottom: The glass tube slipped down the trout's throat.





through which could slide a glass rod. The capsule was put into the distal end of the tube, the tube gently slipped down the oesophagus of the anesthesized fish such that the end was just at the stomach, and the rod used to push the capsule into the stomach. The tube and rod were then removed.

Preliminary analysis of 10 fish showed that immediately after feeding, the capsule was in the stomach in all cases. After 24 hours the capsule was digested. Of almost 8000 capsules force-fed to trout, 7 were regurgitated. This was probably due to incomplete relaxation of the oesophagus because in these instances there was pressure against the glass tube when it was inserted.

2. Voluntary feeding such that a known amount was ingested

In addition to receiving gelatin capsules, the trout were fed Victor Fox pellets. To assure that the experimental fish (force-fed iodide or thyroid hormones) and control trout (force-fed the solvents for the iodide or thyroid hormones) ingested equivalent and known weights of pellets, they were voluntarily fed in the following way. Several pellets were dropped into the water of either the experimental or control aquarium and when the fish consumed these more were offered until 3 to 5 pellets were rejected. The amount ingested was determined and an equivalent amount offered to the second group of fish. The trout voluntarily fed first alternated such that each group (experimental and control) had an equal chance to stipulate the daily food ration.

D. Injections

To compare plasma radioiodide levels following force-feeding to levels following other means of administration, radioiodide was injected intraperitoneally and via the heart. Each injected fish received 0.05 μCi of carrier-free Na ^{125}I and 50 μg of stable iodide (as KI) in double-distilled water. Intraperitoneally-injected fish were given 0.1 ml using a 25 G-5/8" hypodermic needle and heart-injected fish 0.025 ml using a 27 G-1/2" needle.

The fish were anesthesized in 0.005% MS222 for approximately one minute. Intraperitoneal injection was below the right lateral line and beneath the dorsal fin with the needle held at approximately a 45° angle. Heart injected fish were supported ventral side up in anesthetic. The needle was inserted at a slight angle into the centre of the triangle formed by the isthmus between the opercula and the syringe contents slowly expressed.

E. Plasma Collection

Fish were sacrificed by anesthesizing them in 0.01% MS222 for approximately 1 minute. The trout were then weighed to the nearest 0.1 g.

Plasma was collected from the caudal blood vessels either by cutting off the tail or by using a hypodermic needle. Where the tail was cut off, blood flowed freely into an heparinized aluminium weighing dish from where it was transferred via a Pasteur pipet into 1-ml plastic centrifuge tubes and centrifuged (International Micro Capillary Centrifuge, model MB with slant head) at 10,000 to 15,000 x g for 3 minutes. The plasma was pipetted into 2-ml plastic diSPo beakers which were covered with Parafilm and stored at -20° C.

In other fish blood was removed directly from the caudal vessels using 25 G-5/8" hypodermic needles attached to heparinized 1-ml tuberculin syringes. The needle was inserted into the anesthesized fish just posterior to the anal fin and held at a slight angle such that the needle tip rested against the ventral side of the spinal column. When sufficient blood was drawn out from the caudal vessels the needle was removed from the syringe and the blood expressed into a 1-ml plastic centrifuge tube. It was centrifuged as above and the plasma stored as described.

The carcasses of all fish were individually wrapped in paper towels and stored at -20° C.

F. Total Iodide Determinations

The Hycel Cuvette Protein Bound Iodine method (Hycel Inc., Houston, Texas) was used to determine the total iodide.

The major reactions involved are:

(i) Perchloric acid destruction of proteins and the release of bound iodide

(ii) Oxidation of iodide to iodate

(iii) Conversion of iodate to iodide and the reduction of ceric ammonium sulphate by arsenic acid, catalysed by iodide; the rate of catalysis being proportional to the iodide concentration at standard time and temperature.

The procedure involves five steps.

1. Preparation of samples

The given standards were at concentrations of 0, 10, 15, and 20 µg I/100 ml, prepared as KI in water. A volume of 0.1 ml of standard or 0.1 ml of the plasma sample was pipetted into individual cuvettes (19 x 150 mm, Hycel certified cuvettes). In many instances the form of the sample was modified. Brook trout plasma with high iodide levels, which would exceed the range of the standards, was diluted with distilled water and 0.1 ml of the diluted plasma added to a cuvette. Solids (invertebrates and commercial foods) were dried to constant weight (105° C for 24 hours), ground into a powder using a mortar and pestle and a weighed amount added to a cuvette. For analysis of water samples, one litre of water was boiled to 15 to 20 ml and this was centrifuged (Sorval GLG-1) for 5 minutes at 3,000 rpm. One tenth of a ml of the supernatant was analysed. To test the accuracy of this method a tracer dose of ^{125}I was added to the original water sample. Radioiodide recovery was 96.8% for water samples treated in this fashion.

2. Addition of digestion reagent: perchloric acid destruction of proteins and the release of bound iodide.

Two millilitres of digestive reagent (0.025% vanadic acid in 72% perchloric acid) were added to each of the cuvettes. Cuvettes containing liquid samples and digestive reagent were shaken using a Vortex Mixer, those with powder were swirled gently by hand to prevent particles from being left on the glass. The cuvettes were placed at 10-second intervals in the Hycel Heating Block (230° C) under a fume hood. They were left for 6 minutes, removed at 10-second intervals and left 15 to 20 minutes to cool.

When less than 20 mg of powder was used it was indistinguishable after digestion from similarly-treated liquid samples. Greater than 20 mg gave a cloudy solution. This was not used as it would interfere with the later spectrophotometric readings.

3. Addition of ceric reagent: oxidation of iodide to iodate

Two millilitres of ceric reagent (0.6% ceric ammonium sulphate in 27% sulphuric acid) were added to each of the cooled cuvettes from step 2. They were immediately vigorously shaken (Vortex Mixer) to assure mixing of the ceric and digestion reagents which have different specific gravities. As this was an exothermic reaction, the cuvettes were left to stand for 15 to 20 minutes to cool to room temperature.

4. Addition of arsenious reagent: conversion of iodate to iodide and the reduction of ceric ammonium sulphate by arsenic acid, catalysed by iodide; the rate of catalysis being proportional to the iodide concentration at standard time and temperature

This was a colour reaction, the colour being inversely proportional to the iodide concentration.

Two millilitres of arsenious reagent (0.9% arsenic trioxide in 8.2% sulphuric acid) were added to the cuvettes from step 3 at 30-second intervals. The cuvettes were immediately well shaken (Vortex Mixer) and placed in a 37° C water bath for 20 minutes. At 30-second intervals they were removed from the water bath, the external surface dried and the optical density at 420 mμ measured using a Bausch and Llomb 'Spectronic 20' spectrophotometer.

5. Calculations

The optical density of the standards (y axis) was plotted against the corresponding iodide concentration (x axis). This relationship on arithmetic paper was parabolic. The unknown values were obtained from this standard curve and multiplied by the appropriate dilution factor. Powders were calculated as μg per gram dry weight.

G. Total Plasma Protein Concentration Analysis

To determine whether the total plasma iodide levels were related to the total plasma protein concentration, the latter was analysed. The Biuret reagent prepared as indicated by Wannemacher et al (1965) was used to determine the total protein concentration of trout plasma. A volume of 2.9 ml of the Buiret reagent was added to 0.1 ml plasma or 0.1 ml of standard. After 30 minutes, the optical density of this colour reaction was measured at 540 mμ on the 'Spectronic 20' which was previously calibrated with distilled water. Lab-trol (Dade Reagents Inc.) which contained 6.95 g protein/100 ml was diluted by 1/4, 1/3, and 1/2 and used as the standard solution. The optical density of the standards were plotted against the protein concentration to obtain the standard curve (a linear relationship). The protein concentration of the plasma was determined directly from the standard curve.

H. Carcass Analysis

To determine the distribution of force-fed radioiodide within the trout, the per cent dose in the tissues and in the carcass minus the analysed tissues were determined. The ratio of radioiodide in the individual tissues to that in the plasma was also calculated.

1. Per cent dose of radioiodide in the tissues

The frozen carcasses were thawed and individual tissues removed and placed separately in disposable plastic counting tubes. Some 4 N NaOH was added to make a final volume of 4 ml per tube. Each tube was counted twice for 5 minutes using a well-type gamma scintillation counter with a thallium-activated sodium crystal, 2 1/4"x 2 1/4" (Nuclear Chicago DS 202). The standards were prepared by adding to two individual counting tubes, the same radioiodide dose which had been given the trout and otherwise treating them the same as the tissues.

2. Per cent dose in the carcass

The frozen trout minus the tissues removed for individual tissue analysis was sliced with a sharp edged knife. The slices plus 1/3 the fish weight of water were added to a 570 ml stainless steel beaker of the Sorvall Omni-Mixer and homogenized. Some skin and skeleton generally resisted homogenization, but were included with the homogenate mixture. The homogenate was diluted and mixed to make a final volume devisible by 50 ml, and 50-ml aliquots were added to the appropriate number of 100 ml beakers. The uppermost lead shield of the counter (Nuclear Chicago, DS 202 well-detector) was removed, the beakers centred over the crystal and the thick suspension counted. Standards were prepared by injecting intraperitoneally the original dose into 10 trout and immediately sacrificing them. The standard fish were sliced, homogenized and counted in an identical fashion.

3. T/P (tissue/plasma ratio) of radioactivity

The frozen carcasses were thawed. The tissues were removed, weighed to the nearest 0.005 g, placed in disposable plastic counting tubes, diluted to 4 ml with 4 N NaOH, allowed to digest for approximately 1 hour and then counted twice for 5 or 10 minutes. A volume of 0.1 ml of plasma of each fish was counted in a similar fashion. For each tissue the ratio of the counts per minute (cpm) per gram of tissue to the cpm per gram of plasma (T/P ratio) was calculated.

I. Thin-Layer Chromatography

The radioiodide constituents in the plasma following force-feeding of T_4^* were determined by performing thin-layer chromatography on the plasma. A volume of 100 ml of solvent (butanol:ethanol:6 N NH_4OH; 5:3:1 v/v) was added to the chromatography jar (22 x 10 x 21.5 cm) which was lined on three sides with solvent saturated filter paper. The contents of the jar were allowed to equilibrate for 1 hour. The medium (Baker-flex silica gel IB-F, 20 x 20 cm) was divided into three 2" channels by removing the gel medium with a sharp pencil. One-half lambda of each non-radioactive standard (thyroxine, triiodothyronine and reverse-triiodothyronine) was spotted on the 2" channels using 1/2 λ-disosable micropipettes. The reducing agent methyl-mercapto-imidazol, was added to the plasma to keep ^{125}I in the ionic form and prevent it from iodinating plasma proteins and standards. Approximately thirty 1/2 λspots of plasma sample were applied in a line across the 2" channel.

When the solvent had migrated 12.5 cm, the medium was removed from the chamber and dried under a fume hood. The standards were viewed under U.V. radiation and their positions noted. The 2" channels were cut serially into approximately forty 1/8" sections which were cut in half, both halves placed in a counting tube and counted. The cpm of each section were plotted against distance and the Rf values of the resulting radioactive peaks compared to the Rf values of the non-radioactive standards. The per cent of the total radioactivity in each peak was determined.

J. Dialysis

The per cent of the iodide bound to plasma proteins was determined using dialysis techniques. Preliminary experiments to establish the dialysis technique (equilibrium time, reproducibility etc.) herein described are given in Appendix A.

Seven-to-nine-centimeter lengths of dialysis tubing (Union Carbide, size 0.64 cm with pore radius 24 Å) were soaked for approximately 24 hours in double-distilled water, rinsed in fish physiological saline (Huang and Hickman, 1968) and damp dried between two paper towels. A knot was tied at one end, 0.2 ml plasma pipetted into the resulting sac, and the top closed with a slip knot on a 20 cm length of string. The sacs were put into glass tubes (9 x 150 mm) which contained 4 ml of saline and 0.05 μCi ^{125}I. The tubes were covered with Parafilm and masking tape and shaken at 13° C and 250 rpm in a metabolic water bath shaker. After 5 hours the tubes were removed and 0.1 ml of plasma and saline added to separate counting tubes, diluted to 4 ml with 4 N NaOH and counted twice for 5 minutes or 10,000 cpm.

Two modifications to this procedure were used. The first modification involved reversing the plasma and saline. That is, 4 ml of plasma were added to the glass tube outside the sac and 0.2 ml of saline and 0.05 μCi ^{125}I were placed inside the sac.

The second modification involved adding iodide to the saline. The amount of iodide added and the reasons for this addition are given in the Results.

The per cent of the radioiodide bound was calculated as described by Huang and Hickman (1968):

$$\% \text{ bound} = \frac{\text{cpm/0.1 ml plasma} - \text{cpm/0.1 ml saline}}{\text{cpm/0.1 ml plasma}} \times 100.$$

RESULTS

A. The Iodide Content of Several Trout Foods

The total iodide content of five foods fed to brook trout either at the West Hawk Lake Hatchery or at the laboratory was determined (Table III). The Ewos food with 31.3 ± 2.96 to 35.2 ± 1.81 μg I/g dry wt contained 20 to 78 times as much iodide as the other foods examined.

B. The Iodide Content of Several Natural Foods of Brook Trout

Invertebrates commonly found in the stomachs of brook trout (Literature Review) were collected and their total iodide content determined (Table IV). The values ranged from 0.303 - 0.057 μg I/g dry wt (Acroneuria) to 8.57 - 1.03 μg I/g dry wt (Trichoptera cases). The iodide content was less than in Ewos food but except for Acroneuria, the same or greater than in the other foods examined (Table III).

C. The Iodide Content of the Water

Monthly samples of the water supplied to the fish at the West Hawk Lake Hatchery and the Zoology Department were analysed for total iodide content (Figure 2). The iodide levels of the water from the Zoology Department ranged from 1.26 to 2.21 μg I/l. There was a gradual decrease from June

TABLE III. The Iodide Content of Several Trout Foods.

Food	No. of Analyses	% Water	μgI/g dry wt. (± 1 S.E.)
Ewos, size 4 trout pellets (Astra Chemical AB Sodertalje, Sweden)	9 7	7.7 7.2	35.2 ± 1.81 31.3 ± 2.96
Victor Fox 5/16" crumbled pellets (Victor Fox Foods Ltd. Winnipeg)	5 3	7.1 7.4	0.47 ± 0.097 0.42 ± 0.085
Ralstrom Purina #3 Floating Trout Chow (Purina Co., St. Louis Missouri)	6	4.9	1.49 ± 0.139
Gencoe Trout Pellets (Minneapolis)	9	6.6	1.58 ± 0.07
Homogenized Beef Liver[1]	7 7 7 7	70.9 65.1 64.1 66.1	0.47 ± 0.026 0.47 ± 0.026 0.51 ± 0.044 0.39 ± 0.036

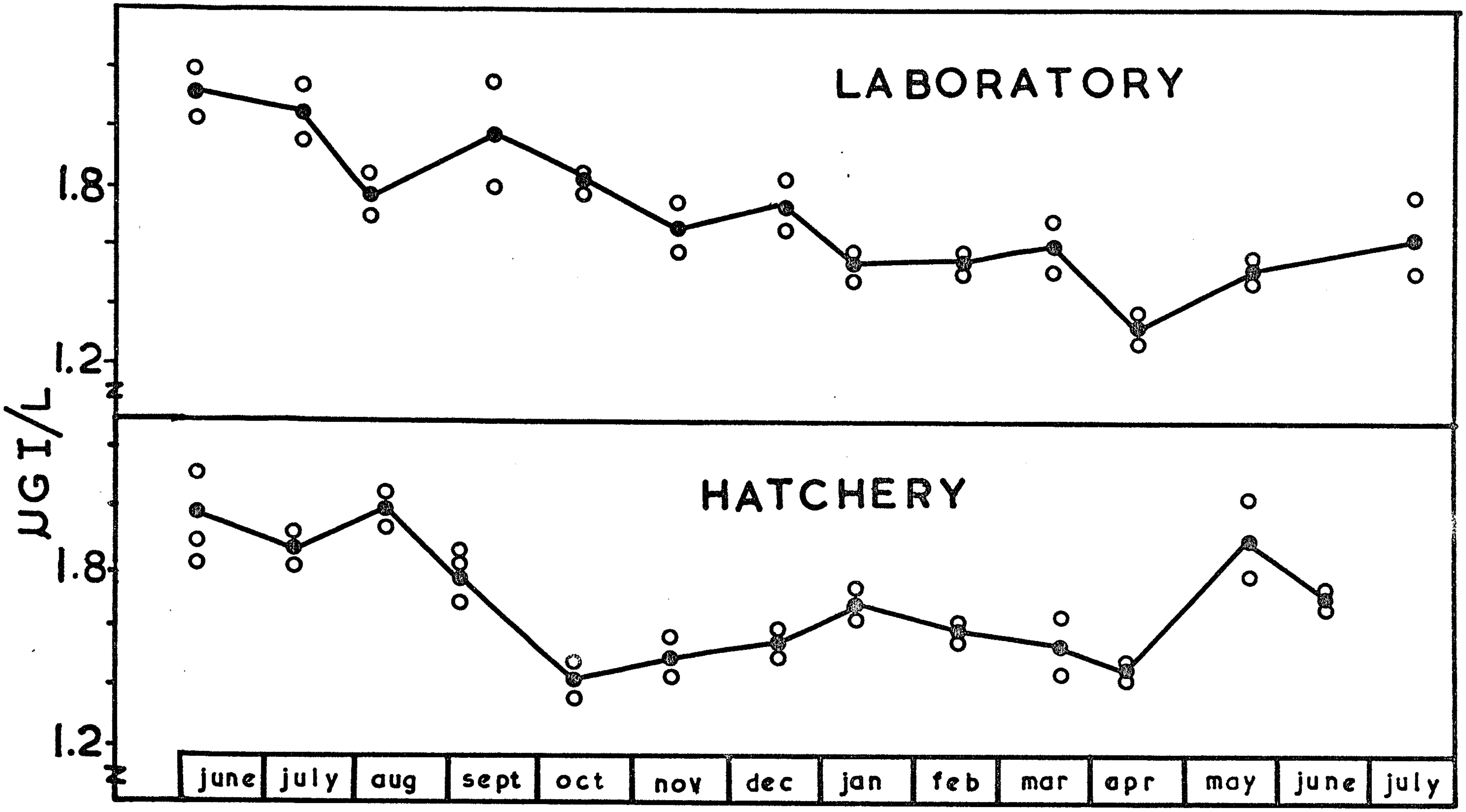
---

[1] The liver was homogenized and frozen as cubes. The cubes were cut into pieces and given to the trout.

TABLE IV. The Iodide Content of Several Natural Foods of Brook Trout.

Food Item	n	mean dry wt per individual (mg)	% Water	μg I/g dry wt. (± 1 S.E.)
Plecoptera				
Acroneuria	4	16.0	64.8	0.303 ± 0.057
Allocapnia	6	258.6	67.8	4.05 ± 0.63
Ephemeroptera				
Heptagenia	6	3.1	73.1	1.98 ± 0.43
Baetisca	7	4.8	71.8	3.35 ± 0.38
Trichoptera				
larvae	7	6.0	80.1	3.37 ± 0.58
cases	5	22.1	68.8	8.57 ± 1.03
Odonata				
Anisoptera (nymph)	6	137.6	56.6	2.87 ± 0.36
Zygoptera (nymph)	6	21.9	-	2.53 ± 0.41
Hemiptera				
Callicorixa	7	4.5	68.9	1.39 ± 0.39
Amphipoda				
Hyalella	2	1.1	59.3	1.15 ± 0.17
Decapoda				
Astacidae	6	109.0 to 2128.5	73.5	4.87 ± 0.29
Coleoptera				
larvae	2	1.85	-	2.25 ± 0.19

FIGURE 2. Iodide content of the laboratory and hatchery water analysed at intervals over a 1-year period. Each point (●) represents the mean of individual samples (o).


LABORATORY
HATCHERY
μG I/L
1.8
1.2
1.8
1.2
june
july
aug
sept
oct
nov
dec
jan
feb
mar
apr
may
june
july

to April but no seasonal trend was evident. The yearly range for water from West Hawk Lake was 1.34 to 2.15 μg I/l and was lowest during the period between October and April. The ranges for the two areas were similar.

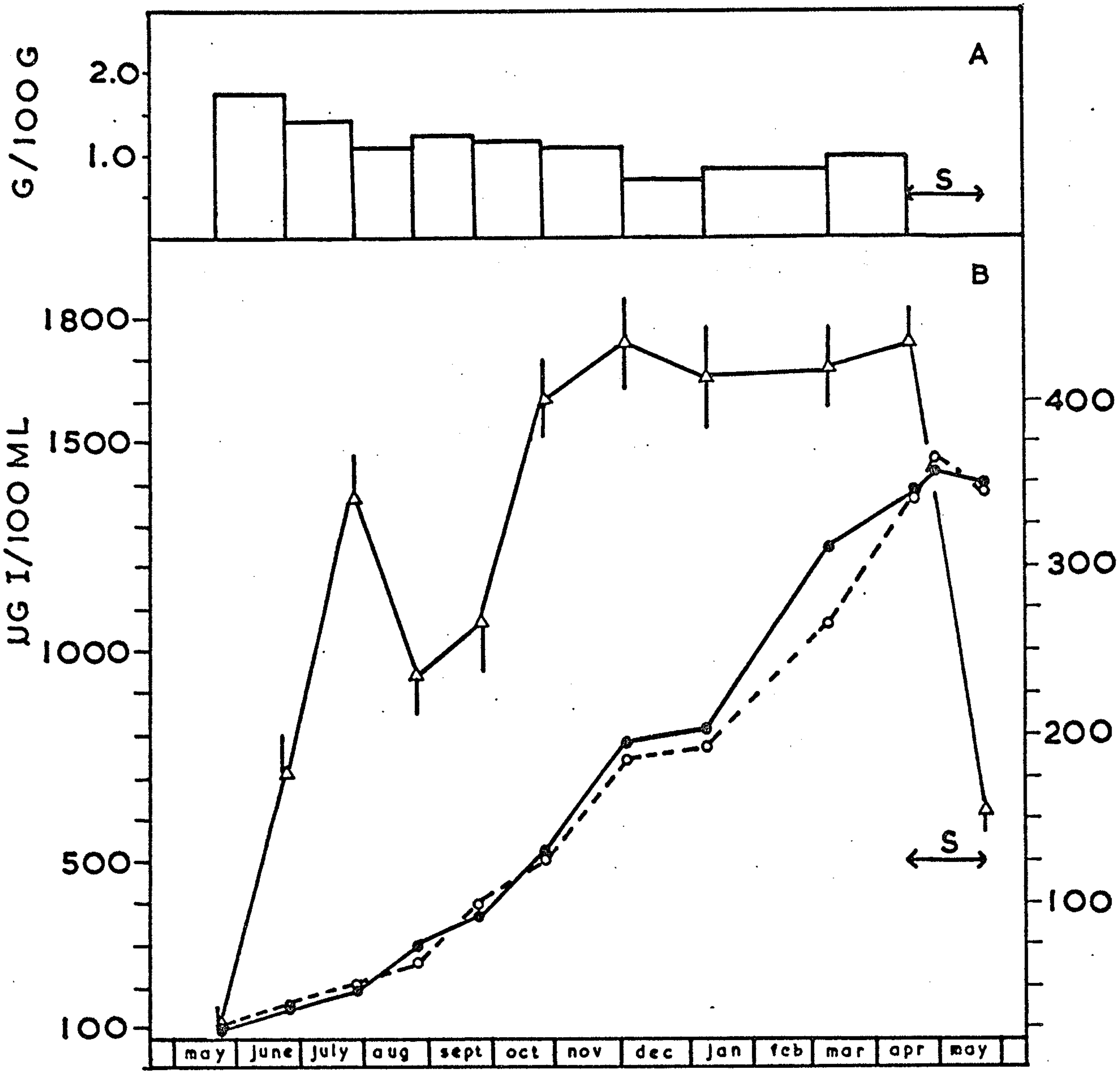
D. Effect of Long Term Feeding of a High Iodide Content Food on Plasma Iodide Levels

On May 25, 1971, 150 trout (approximately 6 months old) were brought from the hatchery and placed in a 125-gal aquarium where they were offered daily Ewos trout pellets. Each month all of the fish were weighed (population body weight), ten fish sacrificed, their plasma collected and total plasma iodide determined. The mean population body weights, sample body weights, plasma iodide levels and amount of Ewos food administered are shown in Figure 3A and B.

The weights of the sampled fish increased up to starvation and were representative of the population except possibly on March 9, 1972. The plasma iodide levels increased 18.9-fold over the first 34 days and continued to increase until 64 days (July 28, 1971). The decrease between July 28, 1971 and August 26, 1971 and between December 2, 1971 and January 11, 1972 may in part be due to the slight decrease in food intake at those times (Figure 3A). The general trend was an increase in the plasma iodide levels to a maximum of 1740 μg I/100 ml on December 2,

FIGURE 3A. Mean amount of food (% body wt) the trout received between successive samples, calculated by dividing the amount of food the fish received each day by the mean body wt mid-way between samples and correcting for a 100 gm fish. S = starvation.

FIGURE 3B. Mean plasma iodide levels (Δ), mean sample body wts (●) and mean population body wts (o) of trout held at 13° C and fed Ewos food daily. Each point represents 10 fish. S = starvation.


A
G/100 G
2.0
1.0
S
B
1800
1500
1000
500
100
µG I/100 ML
400
300
200
100
WT (G)
S
may
june
july
aug
sept
oct
nov
dec
jan
feb
mar
apr
may

1971 with no significant change during the remainder of the feeding regime (April 18, 1972).

Significant decreases ($P > 0.05$) in plasma iodide occurred after both 11 and 37 days of starvation.

The increase in the plasma iodide levels (Figure 3B) corresponded to an increase in fish body weight. To determine whether iodide levels were related to fish size and age three groups of trout, aged one, two and three years, which had been held under uniform conditions, were brought from the hatchery on the same day. They were sacrificed, their plasma collected and total plasma iodide determined. Analysis of variance of the plasma iodide levels of the three groups showed no significant ($P < 0.05$) among group difference suggesting that size and age did not contribute to differences in the plasma iodide levels.

Age (yr)	Wt (g) (± 1 S.E.)	Plasma Iodide (μg/100 ml)
1	29.1 ± 3.9	106 ± 13
2	111.9 ± 6.2	109 ± 16.5
3	507.7 ±14.5	120 ± 20

The sampled fish were sexed and for three groups the gonads were recorded as mature or immature. Within each of these three groups, the plasma iodide levels were ranked in increasing order and the corresponding fish body weight, sex and state of maturity noted (Table V). Comparisons within each group showed that neither body weight nor state

TABLE V. The sex, state of maturity and plasma iodide levels of 3 groups of trout sampled during the long-term feeding experiment. (m = mature; im = immature; * = mature females)

Sample date	Plasma Iodide (µg/100 ml)	Sex	Maturity	Body wt. (g)
Sept. 27/71	430	F	m*	92.2
	505	F	m*	103.3
	790	M	m	108.3
	1035	F	im	105.5
	1050	F	im	76.5
	1085	M	im	85.9
	1150	M	im	92.8
	1370	F	im	80.5
	1650	M	m	186.3
	1700	F	im	90.1
	$\bar{x}$ = 1080 ± 135			
Jan. 11/72	935	F	m*	115.5
	1135	F	m*	160.0
	1450	M	im	116.8
	1640	M	m	253.1
	1675	M	im	204.4
	1780	M	m	143.4
	1800	M	m	295.7
	1800	M	im	116.3
	1904	M	m	180.0
	1909	M	im	201.3
	$\bar{x}$ = 1600 ± 110			
March 9/72	875	M	im	232.2
	1125	F	m*	313.9
	1180	M	m	313.6
	1320	M	m	291.3
	1505	F	m*	141.5
	1555	M	m	405.4
	1875	M	m	215.2
	1875	M	m	291.2
	1970	M	m	223.1
	2030	F	im	262.0
	$\bar{x}$ = 1530 ± 130			

of maturity for males were related to plasma iodide levels. However, immature females had mean plasma iodide levels greater than the levels for corresponding males and in all cases the mature females had plasma iodide levels less than the mean level of that of the 10 fish in the sample.

E. Iodide Absorption from Capsules Force-fed to Trout

Trout fed a high iodide content food (Ewos pellets) in the previous study had significantly increased plasma iodide levels which decreased after starvation. These fish were fed by dropping the stipulated weight of pellets into the water. The quantity of food (and iodide) ingested by each fish was not known. In order to give each fish a known amount of iodide, I decided to force-feed them capsules and to offset any effect of starvation to offer the fish a low iodide content food (Victor Fox pellets).

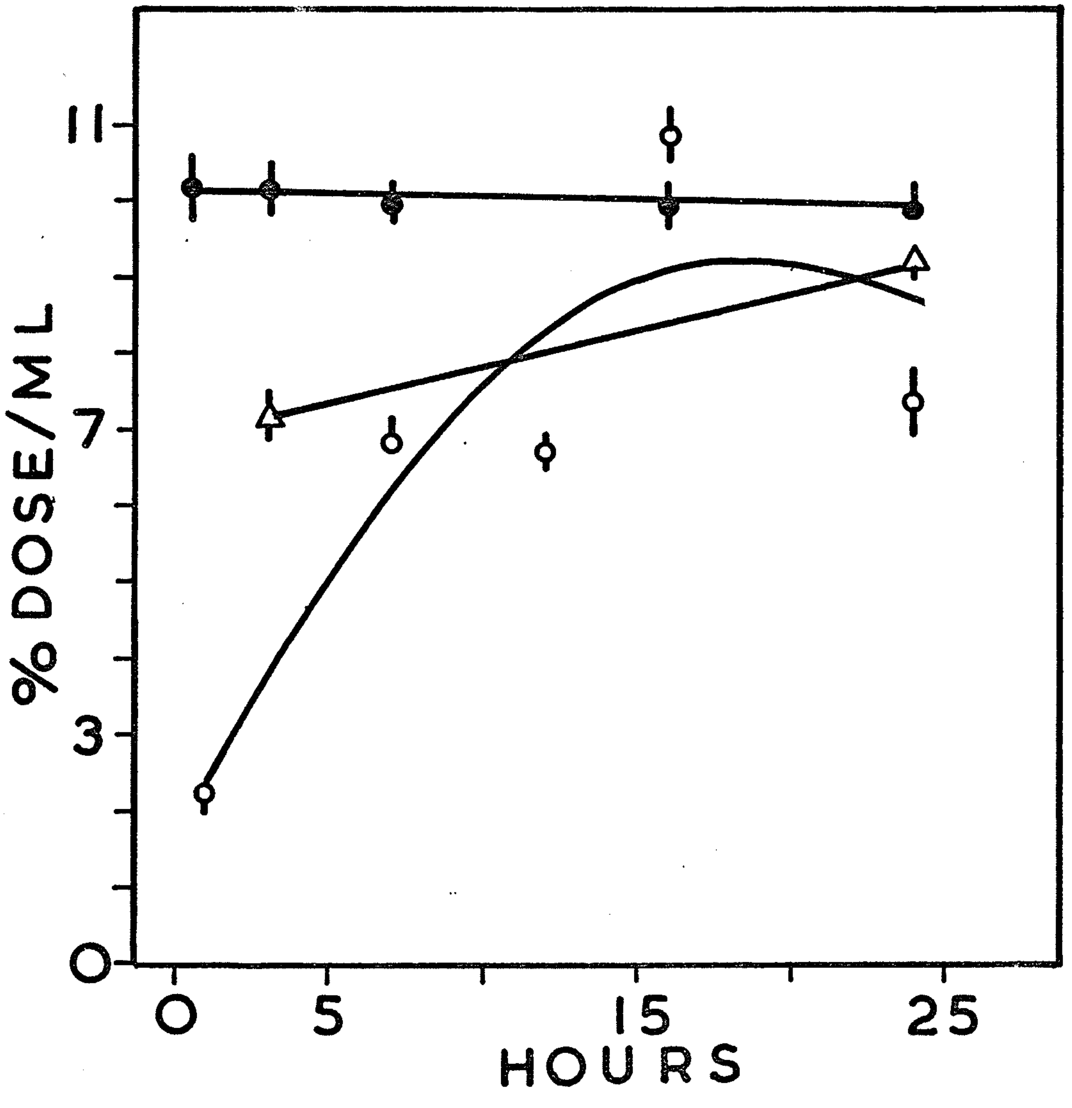
To design such an experiment it was first necessary to determine (i) the efficiency of iodide absorption from the force-fed capsules and (ii) the influence on this absorption of the voluntary ingestion of Victor Fox pellets which could be offered before or after the force-feeding procedure. These variables were examined below by following the uptake of radioiodide from the gelatin capsules.

1. Efficiency of iodide absorption from capsules

Radioiodide uptake from the capsule to the plasma was compared to uptake following intraperitoneal injection and injection directly into the blood stream (heart injection). Each fish received 50 μg I and 0.05 μCi ^{125}I. Twenty trout (mean wt 87.5 ± 6.8 gm) were force-fed gelatin capsules and immediately thereafter voluntarily fed Victor Fox pellets. Three and 24 hours after force-feeding 10 fish were sacrificed, their plasma collected and the per cent dose ^{125}I per ml plasma (corrected for a fish of 100 g body weight) calculated. These sample times were chosen because after 3 hours food was still present in the stomach (and intestine) and after 24 hours the stomach was empty. After 24 hours food was still present in the intestine but no trace of the capsule was visible. The per cent does ^{125}I per ml plasma (corrected for a fish of 100 g body weight) was used as an index of the rate of radioiodide uptake because in brook trout the rate of radioiodide excretion is slow (t 1/2≃10 days) (Higgs and Eales, 1971) and would therefore not influence appreciably the radioiodide levels at the given sample times. Fifty fish (mean wt 95.1 ± 2.5 gm) were injected via the heart and 10 sacrificed and their plasma collected at 1/2, 3, 7, 16 and 24 hours post injection (p.i.). Fifty fish (mean wt 92.1 ± 3.2 g) were intraperitoneally injected and 10 sampled at 1, 6, 12, 16 and 24 hours p.i. The plasma radioiodide values are shown in Figure 4.

The radioiodide level at 1/2 hour post heart

FIGURE 4. Plasma radioiodide (% dose/ml plasma corrected for a fish of 100 g body wt) at different times following heart injection (●), intraperitoneal injection (o), and capsule administration (Δ) of 50 μg I and 0.05 μCi ^{125}I. Each point represents the mean of 10 fish. The bars are ± 1 S.E.


11
7
3
0
% DOSE/ML
0
5
15
25
HOURS

injection was considered as the maximum radioiodide level in the plasma of brook trout at 13° C because this value occurred just after injection directly into the blood stream. The mean radioiodide level, 24 hours after force-feeding was not significantly different ($P < 0.05$) than the level 1/2 hour post heart injection. This suggests that when the stomach is emptied of food and no trace of the capsule remains, the absorption of iodide is efficient. The capsules are therefore a satisfactory method of giving trout a known amount of iodide. Maximum uptake to the plasma from the intraperitoneal cavity was approximately 16 hours and was similar to the maximum following heart injection (1/2 hour) and capsule administration (24 hour).

2. The effect of the timing of voluntary feeding on iodide absorption from force-fed capsules

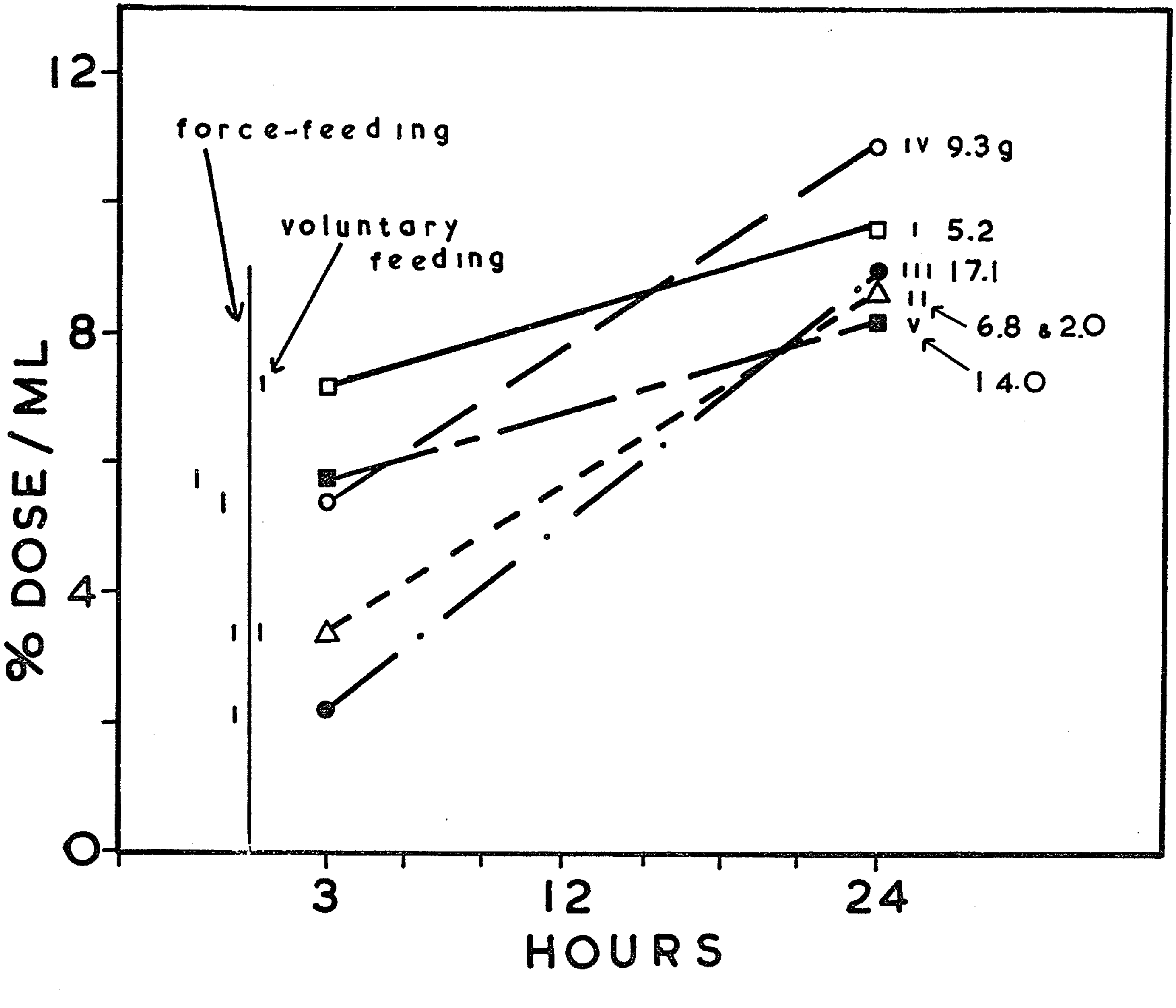
The amount and digestive state of Victor Fox food in the stomach when the capsule was administered would differ if the time between voluntary and force-feeding varied. To determine whether this influenced iodide absorption, five groups, each of twenty trout (mean wt 87.1 ± 8.2 g) were force-fed a capsule containing 50 µg iodide (as KI) and 0.05 µCi ^{125}I. At various times before and after receiving the capsule they were voluntarily fed Victor Fox pellets. Group I was fed voluntarily immediately after receiving the capsule; group II just before and just after; group III just before; group IV one hour before and group V two hours before. The five groups did not voluntarily take comparable amounts of food (Figure 5).

Three and 24 hours after force-feeding 10 fish from each group were sacrificed, their plasma collected and the per cent dose ^{125}I per ml plasma (corrected for a fish of 100 g body weight) calculated. These sample times correspond to those of Figure 4 and were chosen for the same reason.

The presence of food in the gut influenced plasma radioiodide levels measured after 3 hours (Figure 5). However, after 24 hours there was no significant difference $(P < 0.05)$ among the plasma radioiodide levels of the five groups. Thus the residual amount of Victor Fox food and its digestive state when the capsule was administered did not effect the net uptake of radioiodide from the gut to the plasma.

A 100 g fish contains approximately 1 ml of plasma. The per cent of the administered radioiodide dose in the plasma (Figure 5) was 9 to 11% per ml plasma corrected for a fish of 100 g body weight. Therefore only 9 to 11% of the radioiodide dose was in the plasma of the trout. This gives an iodide distribution space of 9 to 11 ml for a 100 g fish and indicates either that appreciable iodide was not absorbed or that iodide had left the blood for the tissues. To account for the extravascular radioiodide, the per cent dose in the stomach, intestine, intestine contents, pyloric

FIGURE 5. Effect of varying the interval between voluntary (Victor Fox pellets) and force-feeding (50 μg I and 0.05 μCi ^{125}I) on plasma ^{125}I levels. Each point represents the mean per cent dose per ml plasma corrected for a fish of 100 g body wt for 10 fish. The numbers on the right indicate the amount of Victor Fox food voluntarily eaten.



force-feeding
voluntary feeding
IV 9.3g
I 5.2
III 17.1
II
V
6.8 & 2.0
14.0
% DOSE / ML
12
8
4
0
3
12
24
HOURS

caecae, thyroid and remaining fish carcass for 5 fish of the five groups was determined (Table VI). There was no significant difference ($P < 0.05$) in the total per cent dose among the five groups of fish. The per cent of the radioiodide accounted for in the trout bodies after plasma collection was 49.3 to 61.2%. Less than 7% of this was in the digestive tract, suggesting that after 24 hours almost all of the ingested radioiodide (and iodide) had been absorbed or egested.

3. Force-feeding of stable iodide for 10 and 20 days

The preliminary experiments indicated that gelatin capsules are a satisfactory method of giving trout a known amount of iodide and that the fish could be voluntarily fed Victor Fox food without influencing the net iodide absorption from the capsules. An experiment was designed in which each fish received a known amount of iodide and to offset any effect of starvation the fish were also voluntarily fed Victor Fox food. Two groups of trout were voluntarily fed (Figure 6B) and force-fed daily. The fish of one group (experimental fish) each received a capsule containing 0.05 μCi ^{125}I and an amount of iodide that they would ingest from Ewos food (50 or 60 μg I, Figure 6A). The control fish were each given a capsule containing 0.05 μCi ^{125}I but no additional iodide.

After 0, 10 and 20 days, all of the fish were

TABLE VI. Per cent ^{125}I dose in various organs and the whole body at 24 hours for groups I-V. Each value is the mean ± 1 S.E. of 10 fish.

Group	Wt. (g)	% Stomach	% Intestine	% Intestine contents	% Pyloric caecae	% Thyroid	% Fish remainder	% Total fish[1]
I	87.5 (6.8)	1.31 (0.17)	0.55 (0.09)	1.52 (0.62)	0.72 (0.18)	2.52 (0.23)	44.1 (1.14)	50.7 (1.7)
II	81.4 (6.8)	2.04 (0.43)	0.50 (0.08)	0.85 (0.29)	0.98 (0.09)	1.86 (0.39)	43.8 (2.24)	49.3 (2.33)
III	94.4 (9.1)	1.89 (0.44)	1.02 (0.17)	2.23 (0.71)	1.06 (0.13)	2.33 (0.22)	52.9 (2.5)	61.2 (2.7)
IV	81.4 (8.6)	2.49 (0.81)	0.60 (0.10)	1.10 (0.31)	0.56 (0.07)	2.69 (0.33)	50.5 (4.4)	57.8 (4.2)
V	90.5 (8.3)	1.11 (0.17)	1.13 (0.14)	2.09 (0.41)	1.11 (0.09)	1.72 (0.17)	50.6 (2.23)	57.8 (1.79)

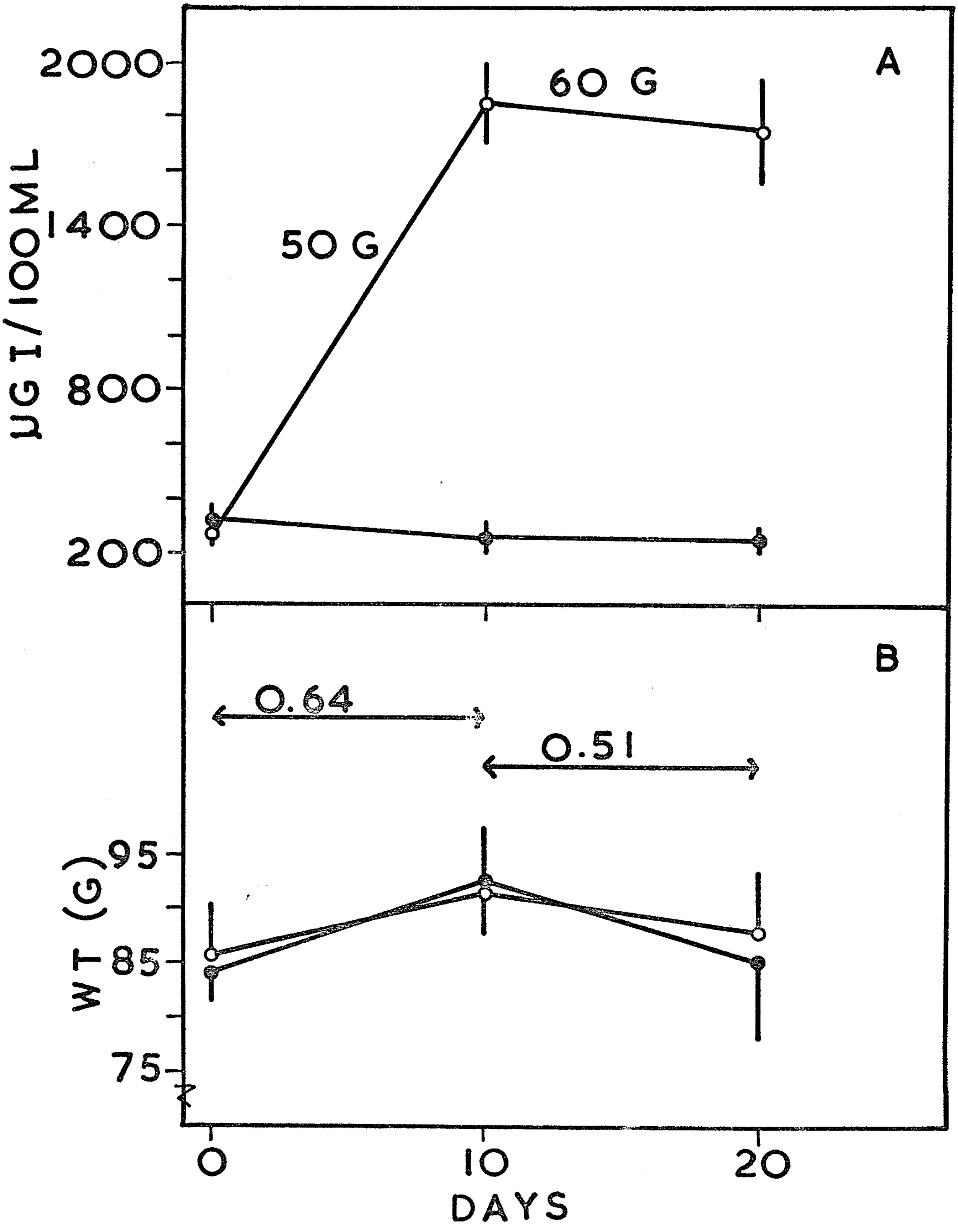
[1] Dose not include approximately 2 ml of blood removed at death nor intestine contents.

FIGURE 6A. Plasma iodide levels of brook trout after 0, 10 and 20 days. Each point represents the mean of 10 fish. The bars are ± 1 S.E.

o: experimental fish (voluntarily fed Victor Fox food and force-fed 50 and 60 μg of I (as indicated) and 0.05 μCi ^{125}I).

●: control fish (voluntarily fed Victor Fox food and force-fed 0.05 μCi ^{125}I).

6B. Mean body weights (gm ± 1 S.E.) of the experimental (o) and control (●) fish. The numbers on the arrows indicate the mean amount (gm) of Victor Fox food voluntarily eaten per day per fish for the 10-day period.



A
B
µG I/100ML
2000
1400
800
200
60 G
50 G
0.64
0.51
WT (G)
95
85
75
0
10
20
DAYS

weighed, 10 fish from each group sacrificed and their plasma collected. The mean population weights and the mean plasma iodide concentrations at the sample times are shown in Figure 6A and B. After 10 and 20 days the experimental fish had significantly greater plasma iodide levels than the control fish and the mean plasma iodide levels of the experimental fish after 10 and 20 days of feeding were similar.

Mean T/P ratios of tissues from the experimental fish were greater than of tissues from the control fish, except for the thyroid (Table VII).

F. The Influence of Alkaline Water on the Plasma Iodide Levels of Trout

High plasma iodide levels influence radioiodide parameters used to study thyroid function (Higgs and Eales, 1971). An experiment was designed to determine whether placing the trout in alkaline water was a possible means of reducing plasma iodide levels. Alkaline water was tested because brook trout held for at least two weeks in aereated water and fed every second day had greatly reduced plasma iodide (Higgs, personal communication). One cause for this effect could have been increased pH due to the build up of metabolites. To assure that the fish had high plasma iodide levels they were force-fed capsules containing stable iodide. Because radioiodide was efficiently absorbed after 24 hours it was thought that stable iodide would significantly contribute to the plasma iodide pool after 24 hours.

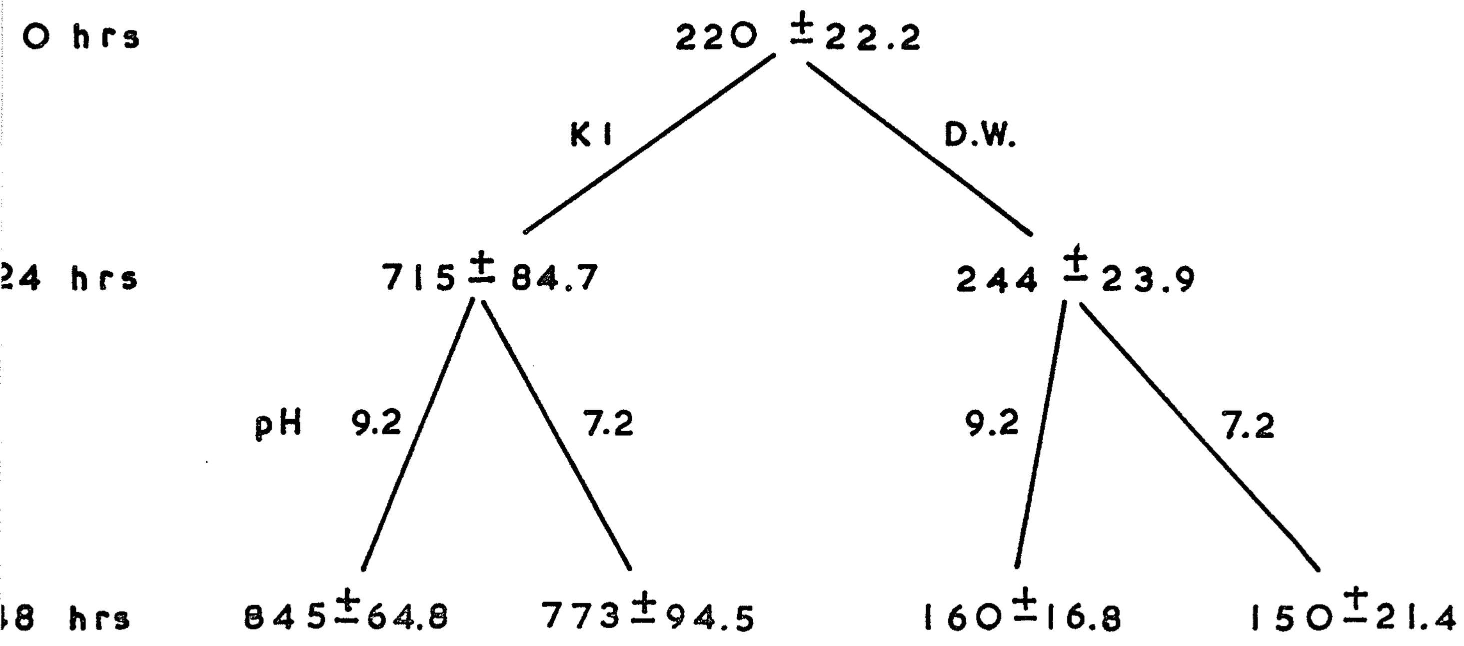
TABLE VII. Tissue/plasma (T/P) ratio of fish sampled 10 and 20 days after eating a controlled amount of iodide.

Tissue	T/P Ratio			
	10 days		20 days	
	Experimental	Control	Experimental	Control
Stomach	0.34 ± 0.021	0.13 ± 0.012	0.36 ± 0.046	0.14 ± 0.012
Intestine	0.24 ± 0.031	0.09 ± 0.006	0.21 ± 0.012	0.11 ± 0.019
P. caecae	0.24 ± 0.032	0.08 ± 0.009	0.21 ± 0.019	
Liver	0.33 ± 0.020	0.13 ± 0.014	0.28 ± 0.005	0.15 ± 0.011
Gallblader	0.24 ± 0.031	0.072± 0.012	0.24 ± 0.022	0.093± 0.016
Kidney	0.16 ± 0.011	0.12 ± 0.019	0.14 ± 0.009	0.09 ± 0.015
Spleen	0.24 ± 0.032	0.07 ± 0.007	0.18 ± 0.019	0.14 ± 0.037
Muscle	0.09 ± 0.009	0.04 ± 0.004	0.09 ± 0.009	0.04 ± 0.001
Fat	0.05 ± 0.010	0.02 ± 0.006	0.03 ± 0.004	0.02 ± 0.002
Gonads	0.24 ± 0.028	0.15 ± 0.040	0.20 ± 0.017	0.12 ± 0.023
Dorsal skin	0.20 ± 0.027	0.15 ± 0.028	0.26 ± 0.045	0.11 ± 0.016
Ventral skin	0.25 ± 0.029	0.16 ± 0.016	0.25 ± 0.016	0.161± 0.023
Air bladder	0.15 ± 0.033	0.09 ± 0.020	0.13 ± 0.030	0.06 ± 0.009
Heart	0.36 ± 0.038	0.20 ± 0.015	0.34 ± 0.015	0.25 ± 0.053
Gills	0.38 ± 0.037	0.18 ± 0.016	0.36 ± 0.023	0.31 ± 0.026
Thyroid	0.47 ± 0.027	0.62 ± 0.130	0.46 ± 0.029	0.79 ± 0.092

The stable iodide uptake 24 hours after force-feeding was examined followed by the influence of alkaline water on the plasma iodide levels. Two groups of 30 fish were force-fed capsules. In one group (mean wt 33.2 ± 3.1 g) each fish received 25 μg iodide in 0.1 ml double-distilled water and each fish in the second group (mean wt 29.8 ± 2.9 g) received double-distilled water. After 24 hours, 10 trout from each group were sacrificed, their plasma collected and the plasma iodide levels determined. Ten fish from each group were then placed in two separate aquaria which contained aereated water at pH 9.2. The remaining 10 fish from each of the two groups were left in the original aquaria (water pH 7.2). After 24 hours the 40 fish were sacrificed, their plasma collected and the total plasma iodide content determined.

The mean total plasma iodide levels for each group of sampled trout are shown in Figure 7. The plasma iodide levels of the fish which were force-fed 25 μg iodide increased 3.3 fold after 24 hours and are significantly greater ($P > 0.05$) than of the trout force-fed the distilled water. This suggests that stable iodide is quickly absorbed from the capsule. There was no significant difference between the mean plasma iodide levels of the fish given the iodide and then placed in either the alkaline water or left in original water and between fish force-fed distilled water and placed in the alkaline or original

FIGURE 7. Plasma iodide levels of trout 24 hrs after being fed 25 μg I (KI) or distilled water (DW) and an additional 24 hrs after being held in alkaline (pH 9.2) or laboratory (pH 7.2) water. Each value is the mean ± 1 S.E. for 10 fish.


0 hrs
220 ± 22.2
K I
D.W.
24 hrs
715 ± 84.7
244 ± 23.9
pH 9.2
7.2
9.2
7.2
8 hrs
845 ± 64.8
773 ± 94.5
160 ± 16.8
150 ± 21.4

water. Alkaline water (pH = 9.2) did not influence the plasma iodide levels and placing brook trout in water at pH = 9.2 for 24 hours is not a means of reducing their plasma iodide levels.

G. Controlled Feeding of MIT, DIT, T_3 and T_4

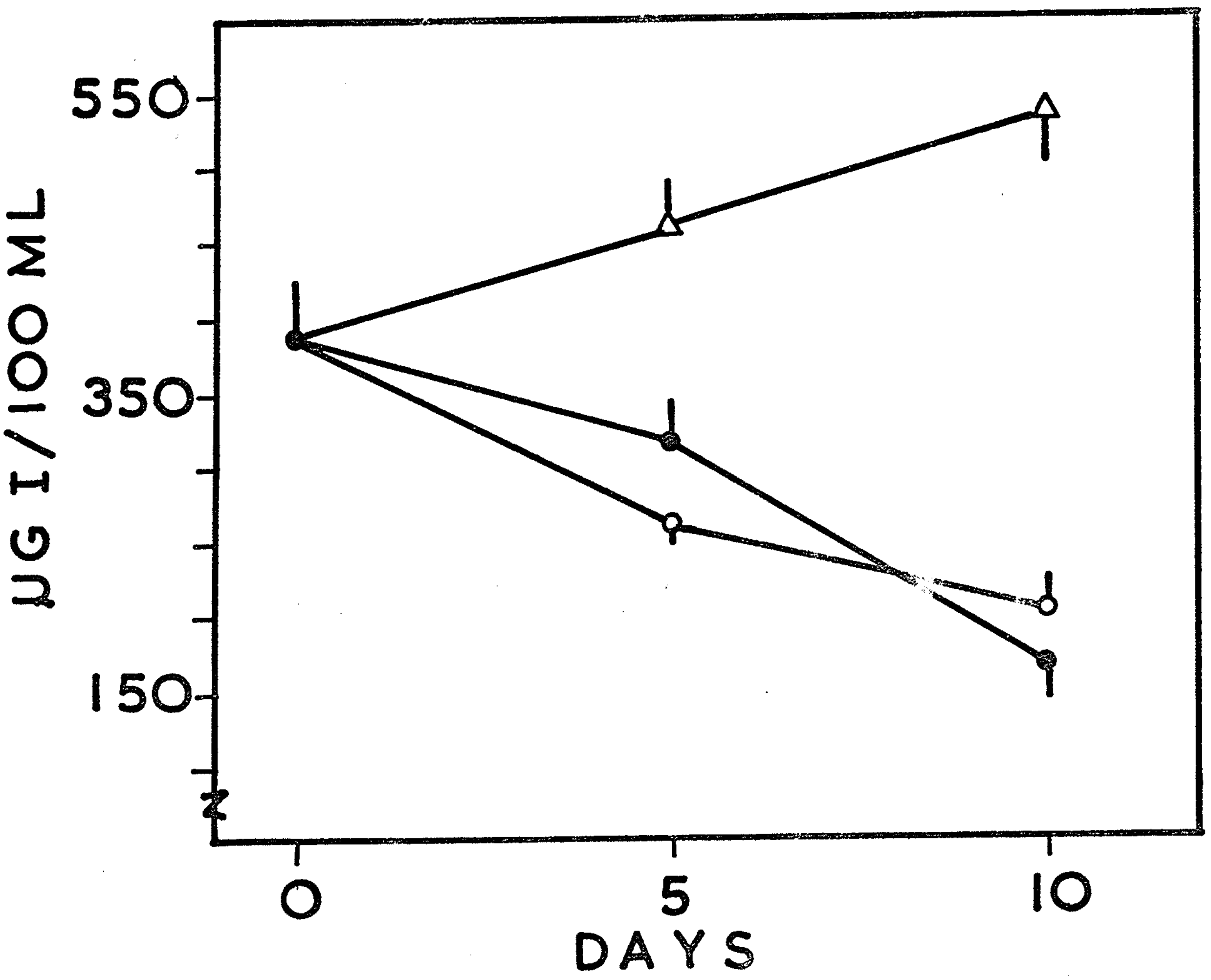
Inorganic iodide was efficiently absorbed from the capsule and contributed significantly to the plasma iodide pool. The natural diet of brook trout includes fish from which the brook trout would obtain organic iodide, predominately from the thyroid. Therefore an experiment was designed to determine whether feeding trout organic iodide in the form of MIT and DIT and T_3 and T_4 contributed to the total plasma iodide pool. The amount given to the trout was that estimated from the organic content of the thyroid of other fish (Table II). Three groups each of 16 trout were voluntarily fed Victor Fox pellets and force-fed capsules daily. The trout of one group (mean wt 161.8 ± 10.1 g) each received a capsule containing 3 µg MIT and 5 µg DIT, those of the second group (mean wt 151.8 ± 11.2 g) a capsule containing 3 µg T_3, 5 µg T_4 and 0.05 µCi ^{125}I - $T_4(T_4^*)$ and those of the third group a control solution (the solvent for the organic compounds).

After 0, 5 and 10 days, eight fish from each group were sacrificed, their plasma collected and the total plasma iodide level for each fish determined. The mean plasma iodide levels are shown in Figure 8. The fish fed MIT and DIT had significantly greater ($P > 0.05$) mean plasma iodide levels than the control fish after 5 and 10 days. The mean plasma iodide level of the fish fed T_3, T_4 and T_4^* were not significantly different ($P < 0.05$) than the mean level of the control fish at the two sample times.

The amount of radioactivity in the plasma of the fish fed T_3, T_4 and T_4^* and sampled after 10 days was extremely low (approximately 200 to 500 cpm/0.1 ml plasma). Thin-layer chromatography of this plasma indicated that the radioactivity was inorganic iodide. Mean T/P ratios of the stomach, intestine contents, liver, gall bladder and thyroid for these fish are tabulated below. The high values observed for the intestine and intestine contents suggested that the T_4 was passed out via the digestive tract. This explained the low amount of radioactivity in the plasma and the negligible effect of feeding, at least the T_4, on the plasma iodide levels.

Tissue	T/P ratio ± 1 S.E.
stomach	0.429 ± 0.11
intestine	7.27 ± 1.82
intestine contents	11.2 ± 4.2
liver	0.207 ± 0.062
gall bladder	0.94 ± 0.29
thyroid	0.318 ± 0.09

FIGURE 8. Total plasma iodide levels of trout receiving 3 μg MIT and 5 μg DIT (Δ), 3 μg T_3, 5 μg T_4 and 0.05 μCi T_4 (o) or control solution (●) daily. Each group was also voluntarily fed Victor Fox food. Each point represents the mean of 8 fish. The bars are ± 1 S.E.

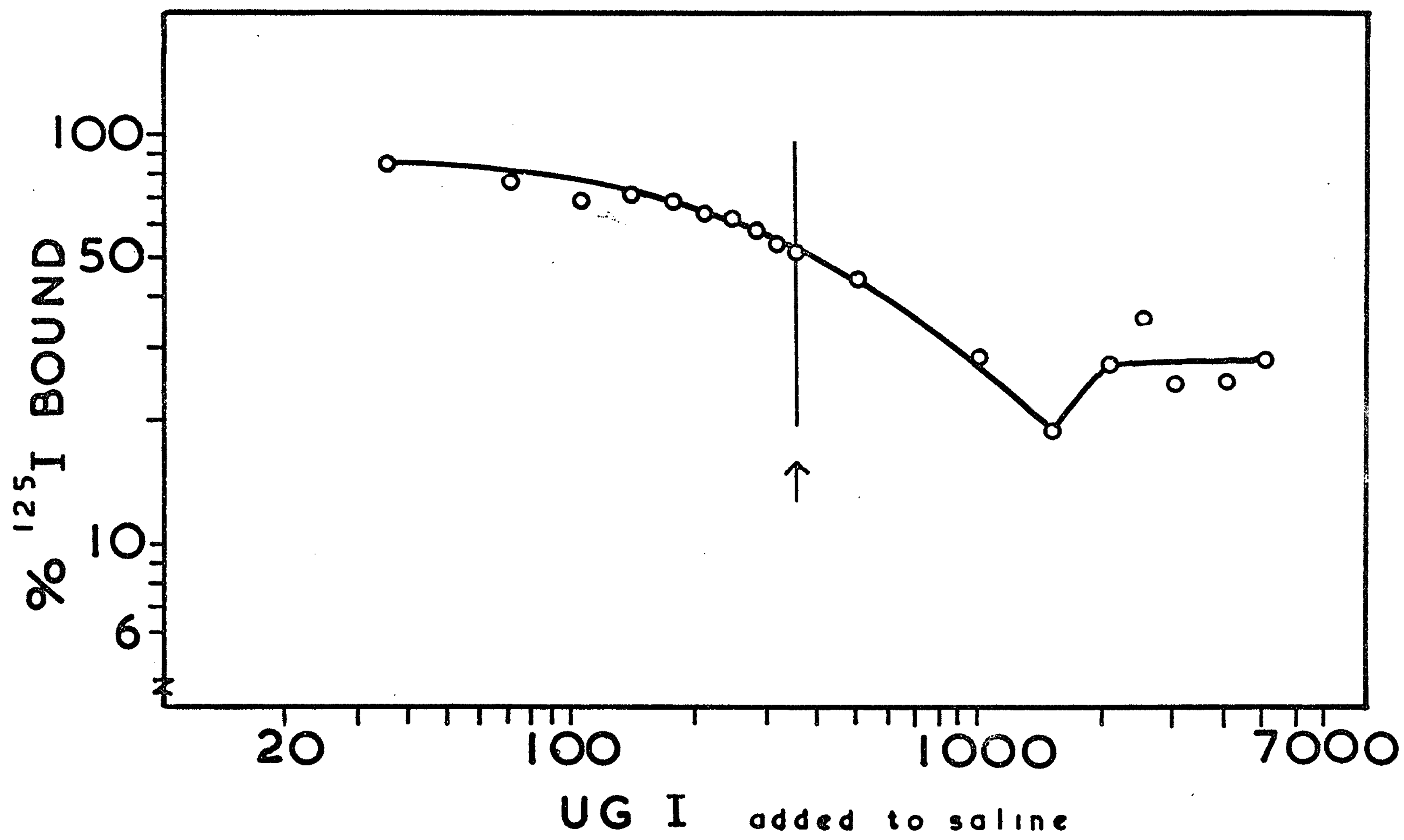

550
350
150
µG I/100 ML
0
5
10
DAYS

H. Investigation of the Relationship Between Plasma Iodide and Plasma Protein Affinity for Iodide Using Dialysis Methods

It is possible that the variation in plasma iodide among fish maintained under identical conditions is due to differences in the plasma protein affinity for iodide. This can be tested using dialysis methods to determine the per cent radioiodide bound in individual plasma samples. However, iodide has a low binding energy (Huang and Hickman, 1968) and therefore care is required in exact application of the dialysis method. For example, when plasma (0.2 ml) was dialysed against 4 ml of saline and 0.05 μCi ^{125}I for 5 hours at 13° C and 250 rpm, 81.6% of the radioiodide was bound in the plasma, whereas when 4 ml plasma was dialysed against 0.2 ml saline and 0.05 μCi ^{125}I under the same conditions, 60.1% of the radioiodide was bound in the plasma. In both cases, however, equilibrium was attained. This discrepancy between the two methods can be explained by iodide movement into the iodide-free saline causing dissociation of the bound iodide followed by further iodide movement into the saline until equilibrium is reached. Thus, at equilibrium, less iodide will be present in the plasma, there will be less competition for binding sites, and a higher proportion of radioiodide in the plasma will be bound. The larger the volume of saline relative to plasma the greater the effect will be. In the first method above, in which the saline volume was 20 times that of the plasma, the iodide movement was greater than in the second method (saline volume 1/20th the plasma volume) and the proportion of radioiodide bound was greater (81.6% vs 60.1%). In fact, due to the small amount of iodide movement in the second method, the 60.1% was considered to be an approximation of the per cent radioiodide bound in this trout plasma.

The above discrepancy would be apparent in plasma samples with different total iodide levels. It can however, be offset by addition of stable iodide to the saline. Figure 9 shows the result of dialysing 0.2 ml of plasma (original ^{127}I content 350 µg I/100 ml) against 4 ml saline to which increasing amounts of iodide were added. The per cent of the radioiodide bound by the plasma decreased when up to 1500 µg I/100 ml were added. This demonstrates the plasma/saline volume difference effect: as the iodide content of the saline was increased less iodide moved into the saline, there was a gradual increase in the amount of iodide remaining in the plasma and a corresponding decrease in the proportion of the radioiodide bound. The addition of 2000 to 5000 µg I/100 ml resulted in an increase and stablization of the per cent radioiodide bound. In this situation iodide was present in quantities greater than the upper limit found for brook trout (1860 µg I/100 ml). The increase in the per cent radioiodide bound was

FIGURE 9. Per cent radioiodide bound to plasma constituents following the addition of increasing quantities of stable iodide to the saline and dialysing 4 ml of the saline containing 0.05 μCi ^{125}I against 0.2 ml aliquots of plasma from a single plasma pool. ↑ represents the plasma iodide content of the plasma.


100
50
10
6
% ^{125}I BOUND
20
100
1000
7000
UG I added to saline

considered as an indication of the affinity of the plasma proteins for iodide in these in vitro conditions.

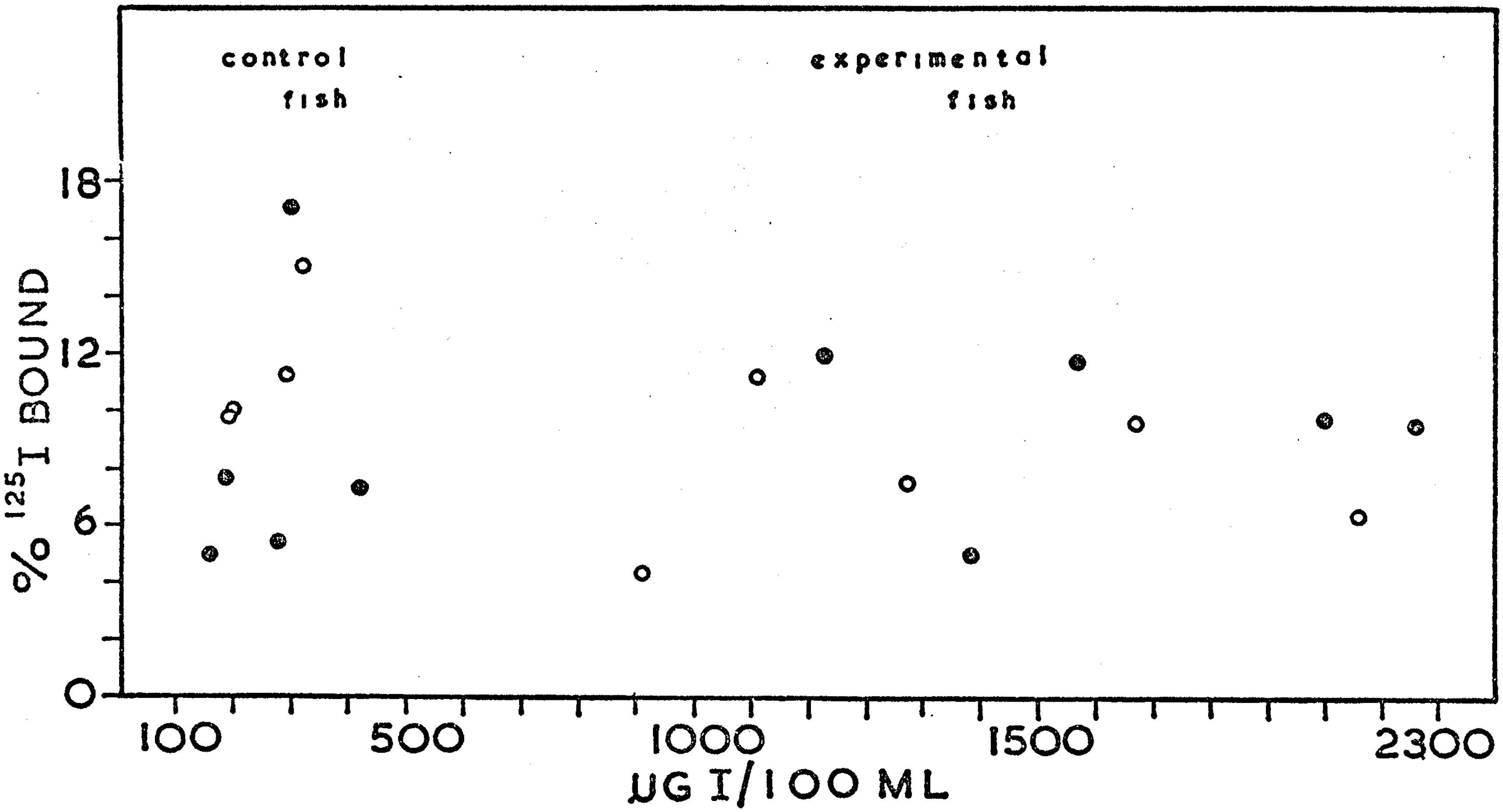
It is therefore clear that if one wishes to compare protein affinity for iodide in fish with varying iodide levels that standardization is necessary. One way to achieve this is to dialyse the 0.2-ml plasma sample against 4 ml saline and 0.05 μCi ^{125}I containing stable iodide at the concentration of 2000 μg/100 ml. This level is greater than that normally encountered in trout plasma and owing to the large volume of saline relative to plasma, would ensure a constant high concentration of iodide (2000 μg/100 ml) throughout the dialysis system for each fish tested. Comparisons of per cent radioiodide bound under these somewhat extreme but standardized conditions should enable any differences in protein-radioiodide (and hence iodide) affinity to emerge.

This procedure was applied to the fish from the 10 and 20 day iodide feeding study. Plasma samples from each of five trout from the four groups (Experimental and Control groups after 10 and 20 days) were analysed (Figure 10). The per cent radioiodide bound was not correlated to the original plasma iodide levels ($P > 0.05$) indicating that the affinity of the plasma proteins was not a major factor determining plasma iodide levels. This conclusion was supported by measurements of plasma protein levels. While protein concentrations ranged from 2.57 to 5.1 g/100 ml

FIGURE 10. The per cent ^{125}I bound (as determined by a modified method of dialysis) vs the plasma iodide levels (μg/100 ml) for each trout from the 10 and 20 day feeding experiment. Each point represents 1 fish.

o: fish sampled after 10 days.

●: fish sampled after 20 days.


control fish
experimental fish
% ¹²⁵I BOUND
18
12
6
0
100
500
1000
1500
2300
µG I/100 ML

they were not related ($P > 0.05$) to the protein affinity for iodide (Figure 11) nor to plasma iodide levels (Figure 12).

FIGURE 11. The per cent ^{125}I bound (as determined by a modified method of dialysis) vs total plasma protein concentration (g/100 ml) for trout from the 10 and 20 day feeding experiment. Each point represents 1 fish.

o: fish sampled after 10 days.

●: fish sampled after 20 days.

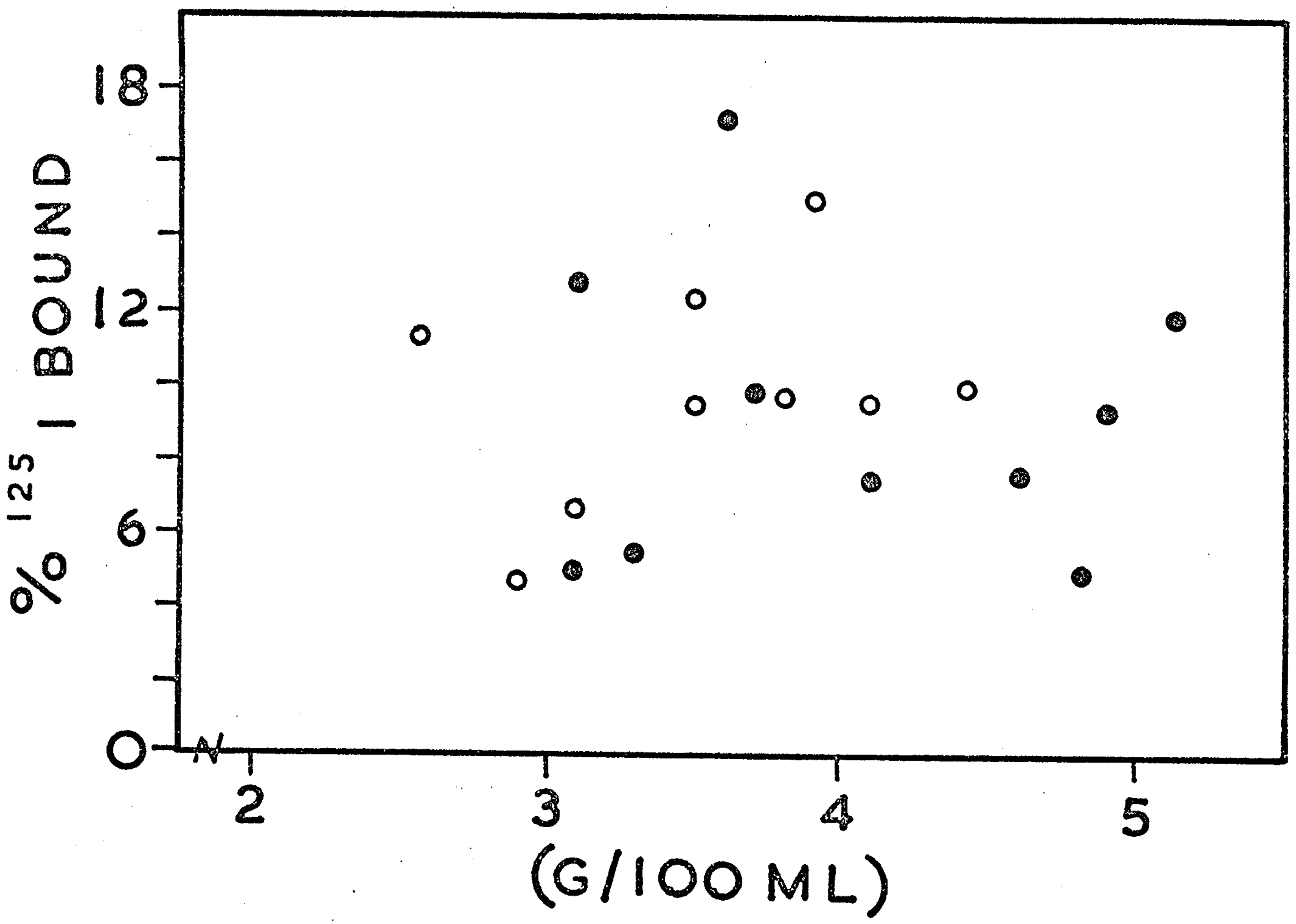
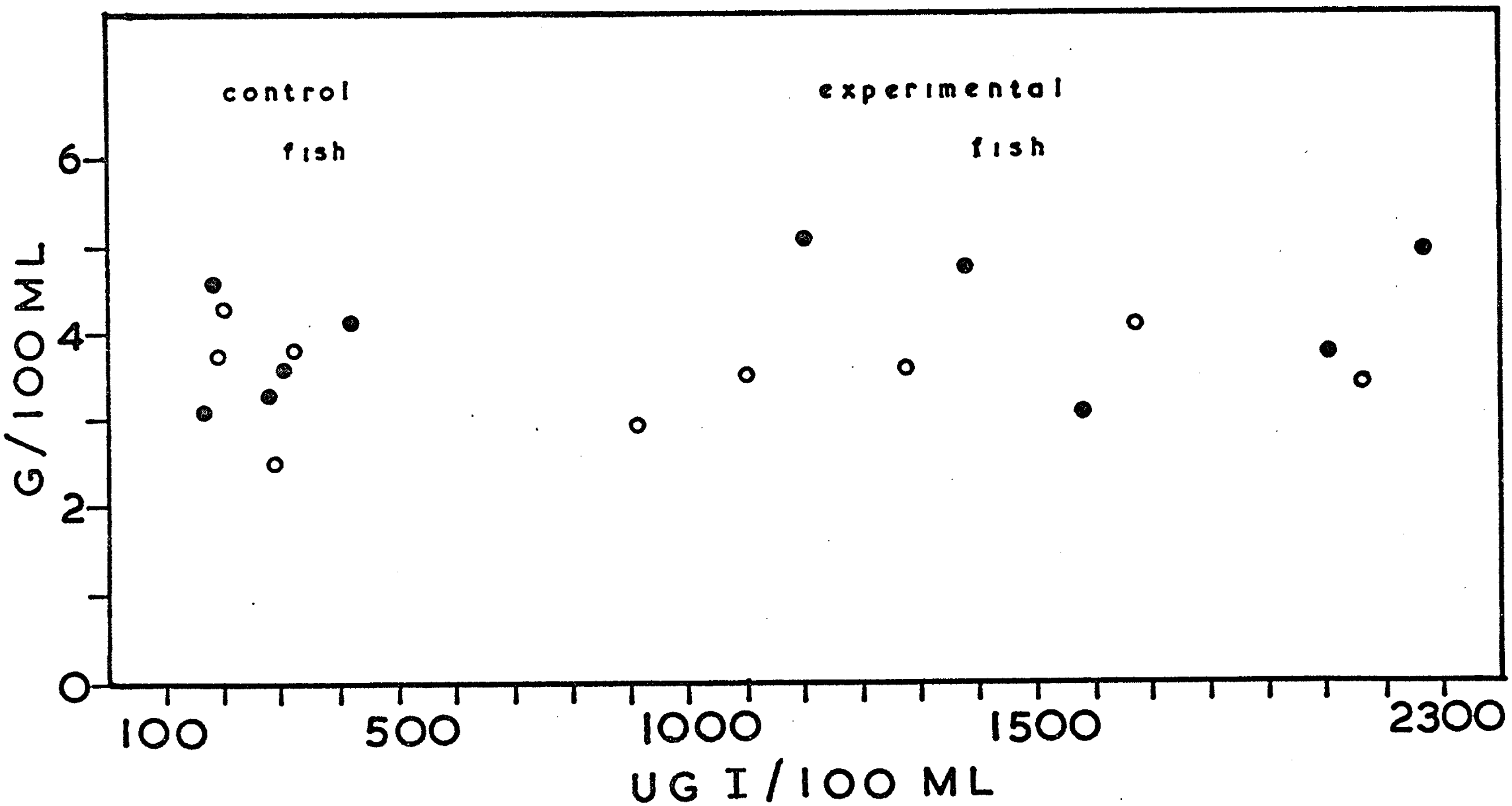

% ^{125}I BOUND
0
6
12
18
2
3
4
5
(G/100 ML)

FIGURE 12. The total plasma iodide vs the total plasma protein concentration (g/100 ml) for the trout from the 10 and 20 day feeding experiment. Each point represents 1 fish.

o: fish sampled after 10 days.

●: fish sampled after 20 days.


control
fish
experimental
fish
G / 100 ML
6
4
2
0
100
500
1000
1500
2300
UG I / 100 ML

DISCUSSION

A. Radioiodide and Stable Iodide Uptake from the Gut

The presence of food (Victor Fox) in the stomach influenced the initial uptake of ^{125}I from the capsule to the plasma. However, 24 hours after the fish were force-fed the amount of radioiodide in the plasma was independent of the amount of Victor Fox food that had been ingested. It therefore appears that it is the total iodide intake and not the amount of ingested food nor the resulting length of time that the food is present which determines the plasma iodide uptake. This would differ if the rate of iodide excretion was fast or varied but in brook trout iodide excretion over 24 hours would be negligible because of the slow rate of iodide excretion in brook trout (Higgs and Eales, 1971).

Plasma radioiodide uptake 24 hours after force-feeding was 9 to 11% of the administered dose/ml plasma and was not significantly different than maximum uptake following injection directly into the bloodstream. Forty-nine to 58% of the radioiodide was in the gastrointestinal organs, thyroid and fish remainder giving a total of 58 to 69% of the administered dose accounted for 24 hours after force-feeding. This suggests that radioiodide is efficiently absorbed from the capsule in the gut. Stable iodide uptake from the capsule was also significant. The plasma iodide levels of trout fed only stable iodide (an amount corresponding to Ewos food) increased 3.3 fold after 24 hours. Plasma iodide was not influenced by placing the fish in alkaline water (pH = 9.2) for an additional 24 hours suggesting that this is not a means of reducing the plasma iodide levels of brook trout.

In conclusion, iodide uptake from the gut lumen to the plasma depends on the total iodide intake and is efficient. The capsule is a satisfactory method of giving fish a known amount of iodide.

B. The Influence of Dietary Iodide and of Starvation on the Plasma Iodide Levels of Brook Trout

Higgs and Eales (1973) observed extremely low thyroxine levels (< 1μg/100 ml) in brook trout plasma. Likewise, Jacoby and Hickman (1966) found low organic iodide levels in rainbow trout. They estimated that 100 g of rainbow plasma contained 4.5 μg of organic iodide (1.1 μg T_4, 1.3 μg T_3 and 1.1 μg MIT and DIT). Therefore the total plasma iodide levels which ranged from 43 to 2300 μg I/100 ml in this study were (except where otherwise stipulated) considered to represent almost entirely inorganic iodide.

Dietary iodide significantly influenced the plasma iodide levels of brook trout. The experimental fish of the 10 and 20 day iodide feeding experiment received an amount of iodide corresponding to what they would ingest from Ewos food (31.3 to 35.3 μg I/g dry wt) and the mean maximum plasma iodide levels observed (1860 μg I/100 ml) were similar to those of the fish fed Ewos food (1740 μg I/100 ml) for 11 months. In both cases the plasma iodide levels reached a plateau in this range suggesting that there is a maximum plasma iodide capacity for these fish at 13° C.

All of the fish studied were held in fresh dechlorinated water (1.26 to 2.21 μg I/l) and the results agreed with LaRoche _et_ _al_ (1965) who found significantly greater plasma iodide levels in rainbow trout in fresh water and fed an iodide supplemented food (6 μg I/g dry wt) for 9 months than in trout held in fresh water and fed the food with no additional iodide.

Starvation for 11 and 37 days resulted in significantly decreased plasma iodide levels of brook trout. This agrees with experiments by Eales (unpublished) who found decreased plasma iodide levels in brook trout which were starved for 29 days relative to trout fed Ewos food for the same period of time. It also supports the observation that reduced food (and iodide) intake per gram of fish per day caused decreased plasma iodide levels.

Prior to starvation the fish were fed Ewos food for 11 months. The mean plasma iodide level for the 10 fish sampled on the day before starvation commenced was 1740 μg I/100 ml and this value was similar to those found for the previous 4 months. However, during this time the fish were fed the high iodide content food (Ewos) daily. Approximately 60% of the plasma iodide is bound to proteins. It therefore appears that daily iodide intake is required to maintain the high free iodide levels. During starvation there would be no additional iodide (except possibly some water iodide) to replenish the iodide stores. Therefore the free iodide could be gradually lost while new free-bound iodide equilibriums were being established.

C. The Possible Iodide Contribution from the Natural Diet of Brook Trout

The food and the water are both potential iodide sources (Literature Review) but which predominates depends on the relative iodide content of each. Therefore an attempt was made to compare the iodide potential in the major food items eaten by brook trout to that in the water from where the food items were collected. The major invertebrate foods contained the same or more iodide than did the laboratory trout foods, except for Ewos pellets. Caddis fly cases had a mean of 8.57 ± 1.03 μg I/g dry wt and account for up to 10% of the stomach contents volume (Ricker, 1930). LaRoche _et al_ (1965) used only 6 μg I/g dry wt in his iodide-supplemented diet. The iodide content of the water from where the invertebrates were collected was

0.98 µg I/l. This quantity is less than in the laboratory water which had little effect on the plasma iodide levels.

Invertebrate iodide is found bound to tyrosines (MIT and DIT) and histidines (MIH) contained within the horny or fibrous structures and to some extent the epithelium (Berg et al, 1955).

Brook trout greater than 6 to 7 inches in length eat crayfish and prey upon fish including small brook trout (Ricker, 1930; Monat, 1965; Lackey, 1969). Crayfish contained a mean of 4.87 ± 0.29 µg I/g dry wt which was more than in the laboratory foods, except Ewos pellets.

From the invertebrates and the prey fish, brook trout would obtain organic iodide. The organic iodide of prey fish would be primarily from the thyroid. Force-feeding T_3 and T_4 to brook trout for 5 and 10 days did not influence the plasma total iodide levels. The T_4 (as indicated by the metabolism of T_4^*) was passed out via the digestive tract. This could have been due to binding of the T_4 to the gelatin capsule (Hays, 1968) but similar results, not using a capsule were found by Sinclair (1972).

Brook trout force-fed MIT and DIT had significantly greater plasma total iodide levels than the control fish after 5 and 10 days. Whether the MIT and DIT were absorbed as inorganic or organic iodide is not known. However, 50% or more of the total iodide of the teleost thyroid tissue (0.1 to 17.87 µg I/100 g fish) is in the form of MIT and DIT (Literature Review) and invertebrates produce MIT and DIT (19 to 96% of the total iodide in insects) and only small quantities of T_3 and T_4 (Berg et al, 1955). Deiodination of these iodotyrosines and absorption of the iodide would supply the brook trout with approximately 0.05 to 9 µg I per prey fish thyroid and 0.06 to 4.7 µg I/g dry wt of aquatic insect.

The natural diet of brook trout has a greater short-term iodide potential than the fresh water. The influence of this diet on the plasma iodide levels of brook trout would not be as great as that of Ewos food but if one assumes efficient absorption of iodide from the natural foods the influence would be greater than that of other laboratory foods. As suggested by Leloup (1970), the gill transport of iodide is most important in fresh water teleosts during starvation.

D. Possible Explanations for the Variable Plasma Iodide Levels

Within groups of 10 fish which were force-fed equivalent amounts of iodide there existed variation in the plasma iodide levels. For example, the plasma iodide levels of trout of the 10 and 20 day iodide feeding experiment which were sampled after 20 days ranged from 908 to 2240 µg I/100 ml. Explanations for this variation include: tissue reserves of iodide; fish age; iodide derived from deiodination of organic iodide compounds; and protein binding of iodide. They are considered below.

Maturing ovaries accumulate iodide (Leloup and Fontaine, 1960; Lindsey et al, 1966; Tarrant, 1971) and mature female brook trout had lower than the mean plasma iodide level in 3 groups of fish examined (Table V). Differences in the state of female sexual maturity partially explained the variable plasma iodide levels observed within groups in the long-term iodide feeding study. However, the trout in the 10 and 20 day feeding study were all immature.

Extrathyroidal and extraovarian tissues also concentrate iodide to a limited extent (Maqsood et al, 1961). Differences in iodide uptake from the plasma to tissues due to differences in organ size or iodide affinity would result in variable plasma iodide levels. Tissue to plasma ratios of tissues from the trout of the 10 and 20 day iodide feeding study were less than one and only slightly variable. It is therefore unlikely that the tissue stores of iodide contribute to the variability in plasma iodide.

Leloup (1970) found plasma iodide levels varied with the age of European eels. In brook trout, the plasma iodide levels were independent of fish age. Also, the fish used in each experiment were of approximately the same age.

Plasma from the trout of the 10 and 20 day iodide feeding study were dialysed under somewhat extreme but standardized conditions in an attempt to measure the relative affinity of the plasma proteins for iodide of the individual plasma samples and to detemrine whether this was related to the variable plasma iodide levels. There was variation in the protein affinity but this was not related to the plasma iodide levels. The total plasma protein concentration was also independent of the plasma iodide levels.

Iodide is produced within fish by deiodination of the thyroid hormones during their degradation (Leloup and Fontaine, 1960). Differences in hormone turnover and rates of excretion could result in differences in deiodination and in the contribution of reabsorbed iodide to the plasma pool. However, the trout in each experiment were under identical conditions (water temperature, water flow, etc.) so it is unlikely that thyroid hormone production and excretion were very variable. Also, deiodination appears to predominate when the biliary excretion route and conjugation are slowed down due to starvation (Eales, unpublished). All of the experimental fish were fed hatchery food so it is unlikely that iodide from deiodination contributed to the variable plasma iodide levels.

In conclusion, there are several possible factors which can contribute to the variable plasma iodide levels. However of the ones examined in this study none were able to explain the observed variation, except the diet which I propose as the most important single factor influencing the plasma iodide levels of brook trout.

CONCLUSIONS

1. Iodide was efficiently absorbed from gelatin capsules and therefore from the gastointestinal tract. The capsules are a satisfactory method of giving trout a known quantity of iodide.

2. Dietary iodide significantly contributed to the plasma iodide levels of brook trout. There appeared to exist a maximum plasma iodide level for brook trout (approximately 1800 µg I/100 ml) at 13° C. Starvation for 11 and 37 days resulted in significantly decreased plasma iodide levels.

3. The natural diet of brook trout contained less iodide than a high-iodide-content food (Ewos trout pellets) but more than four other trout foods examined. A significant iodide source exists in the natural diet of brook trout.

4. Somewhat extreme but standardized dialysis conditions were used to determine the plasma protein affinity for iodide. This was necessitated because the relative volumes of saline and plasma influenced the dialysis technique and this would be apparent in plasma samples with different iodide concentrations. There was no correlation between the plasma protein affinity for iodide and either the total plasma iodide or the total

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APPENDIX A

SOME PRELIMINARY STUDIES ON THE DIALYSIS METHOD

A.1 Iodide Movement Across the Dialysis Membrane

To determine the per cent iodide bound to plasma proteins using dialysis, the free iodide must move across the dialysis membrane, between the plasma and saline. An experiment was designed to test whether radioiodide moved between the two compartments. Twelve dialysis sacs were set up, each with 0.2 ml aliquots of plasma from a single pool inside the sac and with 4 ml of saline outside the sac. Radioiodide (0.05 μCi ^{125}I) was added alternately to the inside and outside compartments of the 12 tubes. The tubes were shaken in a Metabolic Water Bath at 13° C and 250 rpm. After 6, 9, 12, 16, 20 and 24 hours, one tube to which the radioiodide had been added to the inside compartment and one to which it had been added to the outside compartment were removed. A 0.1 ml sample of plasma and of saline were counted. The per cent radioiodide bound for each tube at the sample times is shown in Table A-1. The high (82.0 to 88.4%) per cent iodide bound was found when the radioiodide was added to either compartment. This indicated that the iodide moved across the membrane between the plasma and the saline. In future dialysis the radioiodide was added to the saline.

TABLE A-1. Per cent radioiodide bound following the addition of radioiodide to either the saline or the plasma.

Radioiodide added to	Per cent bound at 6	8	12	16	20	24hr.
Saline	88.4	88.4	85.8	84.1	83.1	85.1
Plasma	87.8	84.5	84.1	82.0	85.2	85.3

A.2 Equilibrium Time and Consistancy Among Replicates

Twenty-four dialysis sacs were set up, each with a 0.2 ml aliquot of plasma from a single pool inside the sac and with 4 ml saline and 0.05 μCi ^{125}I outside the sac. After 4, 6, 8, 10, 16 and 24 hours of shaking in a water bath at 13° C and 250 rpm, 4 tubes were removed and 0.1 ml of plasma and of saline from each tube counted. The per cent radioiodide bound at the sample times is shown in Table A-II. Equilibrium was established at 4 hours and remained until 8 hours. After 8 hours the mean per cent bound values decreased suggesting that the equilibrium was upset. Five hours was chosen as the equilibrium time for future samples.

To determine the consistancy among replicates, 6 dialysis sacs were set up and sampled after 5 hours. The per cent radioiodide bound was 87.4, 88.2, 87.0, 87.4, 87.2 and 87.2 per cent for the 6 replicates. The error in replication was 1.4%.

TABLE A-II. Per cent radioiodide bound following addition of radioiodide to the saline and 4 ml aliquots of saline dialysed against 0.2 ml aliquots of plasma from a single plasma pool for 4, 6, 8, 10, 16 and 24 hours.

	4	6	8	10	16	24hr.
	86.2	86.1	88.1	86.1	84.6	84.0
	86.1	85.7	85.5	84.6	83.0	83.8
	86.0	85.9	85.1	84.8	83.8	83.6
	85.1	84.9	86.0	84.6	85.8	82.0
$\bar{x}$ =	85.7	85.5	85.0	85.0	84.3	83.4

A.3 The Effect of Freezing and Thawing the Plasma on the Dialysis Method

Plasma samples were frozen and thawed various numbers of times before dialysis. It was necessary to determine whether this freezing and thawing altered the per cent iodide bound. A pooled plasma sample was collected and duplicate 0.2 ml aliquots immediately dialysed against 4 ml saline and 0.05 Ci ^{125}I. The remaining plasma was frozen, thawed after 3 days and refrozen. After one week it was again thawed and duplicates dialysed. The per cent radioiodide bound for the fresh plasma was 78.9 and 81.1 per cent and for the twice frozen and thawed plasma 80.8 and 79.5 per cent. Freezing and thawing the plasma did not effect the determinations of per cent radioiodide bound by dialysis.

TABLE A-III. Per cent radioiodide and stable iodide bound following the addition of stable iodide to aliquots of a single plasma pool. Plasma (0.2 ml) aliquots were dialysed against 4 ml saline containing 0.05 μCi ^{125}I for 5 hours.

Plasma iodide (μg/100 ml)	Per cent bound ^{125}I	Per cent bound ^{127}I
650	89.8 (89.7 - 89.9)	91.9 (90.7 - 93.0)
1228	71.9 (71.4 - 72.5)	71.3 (67.8 - 76.3)
1764	70.8 (70.8)	78.4 (77.6 - 79.2)

A.4 A Comparison Between the Per Cent Radioiodide Bound and the Per Cent Stable Iodide Bound

In dialysis, radioiodide was used to determine the per cent iodide bound. The total plasma iodide levels of plasma dialysed were variable and at the high values dilution of the specific activity of the radioiodide could occur. It was therefore necessary to determine whether the per cent stable iodide bound over a range of plasma iodide levels.

A pooled plasma sample which contained 650 μg I/100 ml was divided into three parts. Stable iodide was added to two of the subsamples to give final iodide concentrations of 1228 μg I/100 ml and 1764 μg I/100 ml. Duplicate 0.2 ml aliquots of plasma from each subsample were dialysed against 4-ml of saline and 0.05 μCi ^{125}I. After 5 hours of dialysing, 0.1 ml of plasma and 0.1 ml of saline from each tube were counted and the total iodide content of an equal amount of plasma and saline was determined. The per cent radioiodide bound and the per cent stable iodide bound were calculated using the following equations and are shown in Table A-III.

$$\text{per cent } {}^{125}\text{I bound} \quad \frac{\text{cpm in 0.1 ml plasma} - \text{cpm in 0.1 ml saline}}{\text{cpm in 0.1 ml plasma}}$$

$$\text{per cent stable iodide bound} \quad \frac{\text{total iodide in 0.1 ml plasma} - \text{total iodide in 0.1 ml saline}}{\text{total iodide in 0.1 ml plasma}}$$

The results indicate that the stable iodide measurements are similar to the radioiodide measurements except possibly at the highest plasma iodide level. The results also showed that the per cent iodide bound decreased when the plasma iodide level was increased.

TABLE A-4. Consistency among replicates of total iodide determinations (O.D. = optical density).

Standards		Plasma Samples		Water Samples	
conc.	O.D.	unknown[1]	O.D.	unknown[2]	O.D.
0	1.155 1.187	#1	0.407 0.397	#1	0.323 0.319
5	0.712 0.727	#2	0.601 0.607	#2	0.310 0.292
10	0.400 0.387	#3	0.741 0.735	#3	0.310 0.328
15	0.256 0.267	#4	0.751 0.768		
20	0.198 0.200	#5	0.741 0.735		

[1] The 5 unknowns are 5 individual plasma samples analysed on June 28, 1970.

[2] The 3 unknowns are 3 individual water samples from the same source (laboratory) sampled on June 10, 1970. All were 1 l. samples boiled to 18 ml.