Influence of Iodine Intake on Iodine Distribution in Trout.* (28839)

J. B. HUNN AND E. P. REINEKE
(With the technical assistance of H. W. Kaczkofsky)
Department of Physiology and Pharmacology, Michigan State University, East Lansing

The importance of environmental iodine for normal thyroid function in fish was indicated by the classic report of Marine and Lenhart(1,2) that goiter of brook trout could be alleviated by addition of iodide to the fish hatchery waters. Comparisons between sea-run California steelhead and wild Michigan trout(3) as well as studies on the starry flounder (*Platichthys stellatus*)(4) indicate a close relationship between iodine levels in the habitat and the blood and tissues of the fish. No data have been reported, however, that permit differentiation between iodine supplied by water and food sources. The experiments reported herein were designed to show the effect of water iodide concentration on body stores and distribution of iodine.

Materials and methods. Rainbow trout (*Salmo gairdneri*) were kindly supplied by the Wolf Lake Fish Hatchery, Michigan Department of Conservation, where they had been fed a regular growth diet. Upon arrival in the laboratory they were given a maintenance level of trout pellets, fed on alternate days, except as otherwise stated for particular experiments. They were kept under constant illumination in aged, aerated tap water at 12° ± 2°C, and water was changed every third day.

Protein-bound iodine (PBI) was determined in duplicate by the method of Barker *et al*(6) except that the alkaline fusion was done in nickel crucibles instead of pyrex test tubes. Lower jaws (thyroid) were digested directly with 2 ml 4 N Na_2CO_3 and total iodine determined on aliquots by the same method. Where I-131 was employed, radioactivity was measured on aliquots of the same digests.

To estimate iodine content of tissues other than plasma and lower jaw (Table III) an isotope equilibrium procedure was employed. Nine trout were placed in a tank containing 80 l of tap water with NaI added to bring I^--concentration to 10 μg/l. Water was changed every third day and I^{127} was renewed at that time. Food was withheld throughout the experiment. Each trout was injected daily with 0.1 ml of a solution initially made up to contain 5 μc I-131/ml. One fish was taken for analysis on day 25 and 2 on days 26, 27, 28 and 29. Blood and tissue samples were taken under MS 222 anesthesia. Plasma and lower jaws were analyzed as already described. Other tissues were rinsed in fish Ringer's solution, blotted on filter paper, weighed to the nearest mg and digested in nitric acid. Radioactivity was measured in a scintillation well counter in comparison with appropriate standards.

It was assumed that after the extended period of treatment the various iodine compartments of the body would be in isotopic equilibrium with the PBI. Then:

$$\left(\frac{\mu c\ \text{I-131}}{\text{g of tissue}}\right)\left(\frac{\mu g\ \text{I-127}}{\mu c\ \text{I-131}}\right)=\frac{\mu g\ \text{I-127}}{\text{g of tissue}}.$$

Results. The thyroid of rainbow trout consists of follicles scattered diffusely in loose connective tissue around the ventral aorta. Iodine of the lower jaw is contained principally in these follicles. Thyroidal iodine content (Table I) shows a close relationship to environmental iodine. Hatchery trout receiving iodine principally from food sources had thyroid iodine values about 20 times those of wild Michigan trout. Fasted trout in I^--supplemented water compared closely to sea-run California steelheads which normally have a rich source of iodine in both food and water.

PBI values were also influenced markedly by available iodine. PBI levels of 6.1 and 6.2 μg% of 2 groups of hatchery-fed trout

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TABLE I. Iodine Content of Lower Jaw (Thyroid) of Rainbow Trout.

Type of trout	n	I, μg/l water	μg lower jaw I/100 g body wt Mean	Range
Wild, Mich.*	12	1 or less	.46	.10– .74
Hatchery, normal, food and water	9	1 " "	9.26	4.37–18.99
Hatchery, experimental, fasted	9	10	15.9	10.3 –25.2
California steelhead*	8	50	16.3	11.5 –26.9

* Data from Robertson & Chaney (1953).

were determined in our own laboratory and the Chaney Chemical Laboratory, respectively. Restricted feeding reduced the average value to 1.8 μg%. In iodide-supplemented water, with food withheld, PBI increased to 57.8 μg%.

To facilitate comparison of relative iodine concentration, the distribution values (Table III) are expressed as the calculated iodine content per 100 g of each tissue instead of per 100 g of whole fish. Fasted trout in iodide-supplemented water had an average PBI of 58 μg/100 g plasma. Inorganic iodine comprised 87% of the total plasma iodine. Lower jaw had more than 10-fold the concentration of plasma iodine, but all other tissues were below the plasma level. In these experiments the iodine content of the lower jaw (Table III), calculated on the basis of 100 g of that tissue, was found to be 4.64 mg. Iodine content of the lower jaw was also calculated per 100 g body weight (Table I) so as to be comparable to other published data(3).

Discussion. Detailed studies on iodide transport(7) demonstrate a trout gill mechanism capable of maintaining a plasma:water gradient. However, the present data indicate that the level of iodine stored is closely related to the iodine available in food and/or water. Apparently there are species differences(4) in the extent to which the gill mechanism can maintain iodide levels in fish. It is clear that iodine is readily obtained from food sources via gut absorption or from water via the gills.

Restricted food intake (Table II) reduced plasma PBI, presumably because of lower dietary iodine intake. Fasted trout in iodide-supplemented water had elevated PBI levels and the iodine content of the lower jaw (Tables I and III) was very similar to that of California steelheads that are exposed to ample iodine in both their food and water. Thus it appears that the results of the present experiments are due principally to the environmental iodine supply, and that fasting *per se* had little or no effect on iodine metabolism.

It is of interest that in trout receiving

TABLE II. Plasma Protein-Bound Iodine in Rainbow Trout Reared at Wolf Lake Hatchery.

Feeding schedule	μg I^-/l water	n	μg %, mean ± S.E.
Hatchery-fed	1 or less	4	6.1 ± .54
"	1 " "	10*	6.2 ± .66*
Laboratory-fed alternate days	1 " "	9	1.8 ± .24
Fasted 25-29 days	10	9	57.8 ±4.16
California steelhead†	50	10	21

* Determinations by Albert L. Chaney Chem. Lab., Glendale, Calif.

† Data from Robertson & Chaney (1953).

TABLE III. Estimated Iodine Content of Tissues of Rainbow Trout Exposed to Supplemental Iodine.*

Tissue	μg/100 g tissue mean ± S.E.
Plasma, inorganic	392 ± 50
Plasma, protein-bound†	58 ± 4
Heart	63 ± 9
Gill	57 ± 5
Lower jaw†	4,641 ±552
Liver & gall bladder	118 ± 19
Spleen	52 ± 10
Pyloric caeca	48 ± 5
Head kidney	108 ± 14
Kidney	117 ± 19
Lower gut	61 ± 8
Stomach	52 ± 6
Skeletal muscle	15 ± 2
Eye	43 ± 2

* All trout were fasted 25-29 days and were held in tap water containing 10 μg I^-/l.

† Iodine content determined chemically. All other data were computed by iodine equilibrium procedure.

ample iodide 85-90% of plasma iodine is inorganic, but the PBI level also rises many-fold with increasing iodine supply. Apparently this is due to loose binding of I^- by plasma protein since N-butanol extractable iodine is lower than PBI(4,5).

Both *in vitro* uptake studies(8) and the present *in vivo* data indicate that the only significant iodine concentration in the internal tissues and organs of the trout occurs in the lower jaw, containing the thyroid follicles. Although ovarian tissue was not sampled in this study, it is also known to concentrate iodine(3,9).

Summary. Iodine content of trout tissues is directly related to dietary and/or habitat water levels of iodine. With ample iodide in the water (10 μg/l), trout can extract iodide directly from water and maintain tissue iodine levels comparable to those of sea-run trout. High PBI levels were correlated with high plasma inorganic iodide. An isotopic equilibrium method was applied to estimate the iodine content of trout tissues.

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Behavior of Candida Cells Within Leukocytes.* (28840)

DONALD B. LOURIA AND ROBERT G. BRAYTON (Introduced by E. Alture-Werber)
Infectious Disease Laboratory, Second (Cornell) Medical Division, Bellevue Hospital, New York and Department of Medicine, Cornell University Medical College, New York

We have previously reported that *C. albicans* intravenously inoculated into mice multiply progressively only within the kidneys, and that Candida cells can be visualized within polymorphonuclear leukocytes(1). Therefore, we undertook to analyze the ability of polymorphonuclear leukocytes to phagocytose Candida cells *in vitro* and to study the behavior of the fungus after phagocytosis.

Materials and methods. Six strains of *Candida albicans,* 5 of *Candida tropicalis,* and one of *Candida guilliermondi* were used. Each was maintained by weekly subculture on Sabouraud's glucose agar slants incubated at 30°C. Each maintained a consistent virulence for mice during the experimental period. In virulence studies, groups of 10 Carworth Farms white, male, 17-20 g Swiss mice were inoculated *via* a lateral tail vein with 10^6 to 5×10^6 cells of an overnight culture in a 0.5 ml volume. Mortality was studied for a 30-day observation period. A minimum of 3 experiments was performed on each strain.

Candida cells were harvested in physiologic saline from 24-hour slant cultures, counted directly in Neubauer-Levy hemocytometer, and diluted appropriately in saline. Normal human blood was drawn from an antecubital vein into siliconized syringes and was immediately transferred into siliconized tubes containing 100 units of heparin per 10 ml of blood. Two ml samples were transferred into smaller tubes and Candida cells were added in a 0.1 ml volume so that there was approximately a 2 to 1 Candida to polymorphonuclear leukocyte ratio. The size of the inoculum was determined by standard pour plate enumeration techniques. The blood-Candida

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