

THE COMPARATIVE EFFECTS OF DESICCATED PARROT FISH THYROID GLAND AND SYNTHETIC THYROXIN ON THE RESPIRATORY METABOLISM OF WHITE MICE

RALPH A. KENNER, RICHARD G. BJORKLUND, AND EDGAR C. GASDORF
Bradley University, Peoria, Illinois

ABSTRACT.—Desiccated parrot fish thyroid extract, though questionably calorogenic in fish, has a finite calorogenic effect paralleling that of synthetic thyroxin in mice. The dosages of hormones used in this experiment are apparently pharmacologically effective.

Recent literature concerning the physiology of the thyroid gland in fish reveals that its function is not clearly understood (Pickford and Atz, 1957; Gorbman and Bern, 1962).

The literature does establish that the thyroid hormones of fish are chemically similar to those of other vertebrates. Furthermore, biochemical function in the fish thyroid closely parallels biochemical function in the mammal, although details of the way in which thyroxin enters the chain of biochemical reactions are not entirely clear even for mammals. However, investigators have not always been careful to separate responses to massive effective doses of thyroid hormones from responses to small effective doses of thyroid hormones, thereby providing misleading information as to thyroid function in the species receiving the hormones. Furthermore, it seems most likely that in vertebrates in general, species variation is related to a plurality of tissue responses rather than to a plurality of thyroid hormones.

Data about the effect on fish or other species of vertebrates of thyroid extracts derived from the glands of fish are scarce, owing in part to the unencapsulated nature of the gland in most species. Sembrat (1927) and Mathews and Ash (1951) have used fish thyroids to induce premature metamorphosis in frog tadpoles. We have had some success in repeating the latter's experiments, the preparation of parrot fish thyroid gland being the same one used in the experiment detailed below. Smith and Mathews (1948) reported that parrot fish thyroid increased oxygen consumption in white grunts, *Haemulon plumieri*, over 15 cm in length. However, this work was questioned by Matty (1957) who found that surgical thyroidectomy of the encapsulated gland of the Bermuda parrot fish, *Pseudocrenis guncamata*, did not alter its oxygen consumption. Injection of L-thyroxin or an extract of parrot fish thyroid gland did not change the standard rate of oxygen consumption in intact animals. To our knowledge the work of Smith and Brown (1952) and Matty (1954) are the only reports of a calorogenic effect of fish thyroid extract in a mammal.

One of us (R.G.B.) has been studying thyroid function in goldfish,

Carassius auratus, using both synthetic thyroxin and triiodothyronine as well as desiccated parrot fish (*Pseudoscopus guacamaia*) thyroid. Inasmuch as synthetic thyroid hormones produced marked growth and morphogenic responses in goldfish, while desiccated parrot fish thyroid failed to elicit any kind of response in goldfish, an experiment was conducted to test the efficacy of the parrot fish thyroid preparation used. Results of the experiment are reported in this paper.

MATERIALS AND METHODS

Eleven, adult male white mice, chosen from a stock conditioned to laboratory life for several weeks prior to the start of the experiment, were toe-clipped for individual identification. After random assignment of three animals to a saline control group, four to a group to receive sodium L-thyroxin pentahydrate (Mann Research Laboratories Inc.), and four to a group to receive desiccated parrot fish thyroid, determinations of their relative oxygen consumption were taken by the method of Lauber (1962). The term "relative" is used since the absolute value of the quantity of oxygen consumed is not recorded on the kymograph drum used.

Since conditions remained reasonably constant throughout the experiment, any inherent error produced by the apparatus should be constant. The temperature within the animal test chamber was maintained at $29 \pm 1^\circ$ C. The test chamber was covered with a dark cloth and no significant differences were obtained in repeating observations on animals tested twice within an hour.

All determinations were made between mid-morning and early afternoon, after which the animals were fed a standard pelleted diet. Food, but not water, was removed from the cages during mid-evening at least 12 hours prior to the next series of observations.

The arithmetic means of the last three of four pre-experimental 10 minute determinations for each mouse were used as "basal" rates (Table 1). In this way all animals served as their own standards of comparison in addition to having the saline injected controls available as common standards during the actual experiment. Values in Table 1 represent kymograph readings in mm/gm body weight of mouse per 10 minutes. Data were tested for significance using the H test of Tate and Clelland (1957, pp. 109-111) with the level of significance, alpha (α), chosen as 0.05.

The parrot fish glands were ground to a powder and the powder passed through No. 25 silk bolting cloth. Both parrot fish thyroid powder and thyroxin were dissolved in 0.1N NaOH, neutralized with 0.1N HCl and diluted to the desired concentration in buffered saline adjusted to pH 7.2. Thyroxin (3.2 μ g/gm body weight) or parrot fish thyroid (260 μ g/gm body weight) was injected intra-abdominally. All animals including controls received 0.06 cc of fluid per gm body weight. Relative oxygen consumption of all animals was determined on days 1, 2, 3, 4, 5, 7, 10, and 12 following the single injection and all animals were sacrificed using ether, and carefully necropsied on day 13.

TABLE 1.—MEAN Basal and Post Injection Relative Oxygen Consumption, in Millimeters per Gram Body Weight per Ten Minutes, of Individual Mice Treated with Thyroid Hormones

| Treatment | Mean Basal | Days After Injection | | | | | | | |
|---------------------|------------|----------------------|------|------|------|------|------|------|------|
| | | 1 | 2 | 3 | 4 | 5 | 7 | 10 | 12 |
| Saline | .575 | .509 | .543 | .571 | .580 | .590 | .572 | .568 | .586 |
| | .498 | .540 | .520 | .550 | .511 | .491 | .477 | .476 | .508 |
| | .522 | .545 | .532 | .552 | .515 | .534 | .518 | .514 | .499 |
| Parrot Fish Thyroid | .484 | .514 | .495 | .582 | .698 | .710 | .601 | .537 | .473 |
| | .492 | .502 | .553 | .552 | .727 | .635 | .746 | .493 | .487 |
| | .452 | .540 | .617 | .811 | .624 | .732 | .889 | .484 | .451 |
| | .522 | .511 | .642 | .881 | .674 | .811 | .617 | .584 | .511 |
| L-Thyroxin..... | .480 | .507 | .749 | .813 | .725 | .761 | .629 | .593 | .476 |
| | .492 | .592 | .704 | .687 | .558 | .593 | .580 | .415 | .509 |
| | .506 | .525 | .681 | .684 | .610 | .715 | .680 | .471 | .500 |
| | .452 | .556 | .643 | .750 | .589 | .605 | .624 | .454 | .495 |

RESULTS

The relative oxygen consumption of treated mice is summarized in Table 1. Differences for days 1, 7, 10, and 12 are not statistically significant. Differences for days 2, 4, and 5 are significant and for day 3 are highly significant.

Responses to thyroxin and fish thyroid extract were quite similar, in both magnitude of change and duration of change. The onset of responses lagged slightly in the mice treated with parrot fish thyroid.

DISCUSSION

The presence of a calorogenic effect of a teleost thyroid extract injected into mice parallels the finding of Smith and Brown (1952) for teleost thyroid extract injected into the laboratory rat, and of Mally (1954) for shark thyroid extract injected into laboratory rats.

Necropsy findings in rats treated with fish thyroid extract have not

been reported. It was surprising to find a definite ulceration of the stomach in one of the four animals in each of the experimental groups, but no visible changes in the visceral organs of members of the control group. Two of the remaining three animals in each group showed signs of developing ulcers of the stomach. Hair erection was noted immediately after injection in all groups. Turner (1955, p. 161) states that erection of hair is a sign of "emergency function" mediated by the sympatho-adrenal system. Hair erection disappeared in a few hours in control animals, but persisted approximately 24 hours in the experimental animals.

No sign of puncture of vital organs was evident in any animal and the ulcerations of the stomach wall were interpreted as part of the General Adaptation Syndrome, suggesting that our dosages of hormones were pharmacologically effective. Inasmuch as the dosages used in the present experiment with mice were

comparable on a body weight basis to those used in rats by Smith and Brown (1952), it appears likely that doses used by them were also pharmacologically effective. Heming and Holtkamp (1953) considered anything over 1.5 $\mu\text{g}/\text{gm}$ body weight per day of thyroxin (Smith, Kline, and French Lab. Philadelphia, Pa.) pharmacologically effective in rats.

ACKNOWLEDGMENTS

Thanks are due Dr. William B. Sattelle Jr., Director, and Dr. David W. Menzel, Assistant Director, of the Bermuda Biological Station for Research Incorporated, for collecting the parrot fish thyroid glands.

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Manuscript received January 26, 1963.