

Further Evidence for Thyroid Function in Anuran Larvae

CHARLES H. STEINMETZ¹

Indiana University²

Preliminary observations made on the effects of 2-thiouracil on *R. clamitans* larvae (4) indicated that the thyroid gland of Anuran larvae is actively secreting early in development and that this secretion has a definite role in the growth of the organism. The present paper is an extension of the earlier work and is designed to present further evidence for the function of the larval Anuran thyroid gland.

First, it must be admitted that the earlier experiments did not conclusively demonstrate that the effects of thiouracil were due to an action of this compound resulting in suppression of thyroid function. If such a suppression actually occurs, the results obtained should be reversible by thyroxine treatment, and larvae maintained on a low dosage of thiouracil, i.e. one that would not inhibit normal metamorphosis, would not differ significantly from the controls. Second, there is the possibility that the results obtained on *R. clamitans* larvae are not applicable to other Anuran species that have a free-living larval stage. Finally, the bilateral asymmetry of the gonads of the *R. clamitans* larvae, as determined by the greater length of the left gonad compared with the right in microscopical sections, suggested that if the left gonad of the adult were found to be larger than the right, then somewhat more confidence could be placed in the differences observed between the gonad lengths of the thiouracil treated and control larvae. This situation has been reported to exist in *R. sylvatica* by Hoskins and Hoskins (1).

Materials and Methods

The larvae employed in these experiments were obtained by induced ovulation (3). Mature *R. pipiens* frogs were obtained locally from an animal dealer and the *R. clamitans* and *R. catesbiana* frogs were captured personally at Yellowwood Park. Three separate groups of animals will be considered in this paper.

The first is a group of *R. pipiens* larvae reared in eight-inch finger bowls by a method previously reported (4). Two bowls served as controls while two bowls were treated with 0.030% 2-thiouracil. One group of both the control and thiouracil treated larvae were weighed on the 22nd day after fertilization. Samples of ten body weights were taken on the 35th and 40th days. On the 40th day five animals from each

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²Contribution No. 476 from the Department of Zoology, Indiana University, Bloomington, Indiana.

group were fixed in Bouin's fixative. Also on the 40th day, thyroxine treatments were begun on one bowl of the control animals by adding one cc. of a 0.0010% solution of the culture medium to make a concentration of ten gamma per liter, and the thiouracil dosage was reduced to 0.010%. On the 53rd day five animals from each group were weighed and fixed in Bouin's. Serial transverse 8 micron sections were made of the animals fixed in Bouin's and the length of the gonads was determined by counting the number of sections in which a definitive portion of the gland appeared.

The second is also a group of *R. pipiens* larvae. Fifty larvae were reared in each of two aquaria containing eight liters of aerated tap water. One group was used as a control while the other was treated with a low dosage of 2-thiouracil, 0.0075%. These animals were fed a diet of Pablum supplemented by commercial dog food. A sample of ten weights was taken on the 63rd day. On the 112th day, after approximately 50% of the animals in both groups (17 controls and 25 thiouracil treated) had undergone metamorphosis, the animals were sacrificed and the body weights of the metamorphosed animals were determined.

The third set of animals to be considered is a group of mature male frogs captured on June 2, 1951. These animals were sacrificed shortly after capture and the right and left testes of ten male *R. catesbiana* and twenty-four male *R. clamitans* were individually weighed on a torsion balance to the nearest 0.5 mg.

Results

The results obtained from the *R. pipiens* larvae reared in the finger bowls are summarized in figure 1. The mean body weights of the various groups at 53 days are: control, 118.8 mg.; thiouracil, 169.8 mg.; thyroxine, 45.0 mg. The differences between these means are significant at the 1% level when tested by the "t" test. Before 53 days there is a higher probability that the differences observed are due to sampling error. This is not surprising inasmuch as it was at about this period of ontogeny that significant differences between the thiouracil treated and control series of *R. clamitans* larvae were noted (4), and perhaps this indicates that the thyroid gland of *R. pipiens* begins to secrete somewhat later in ontogeny. The fact that thyroxine reverses the effect of thiouracil strengthens the contention that the results of thiouracil treatment are due to hypofunction of the pre-metamorphic Anuran thyroid gland. The level of thyroxine treatment employed in this experiment resulted in considerable metamorphic advance during the period of treatment, e.g. forelimb emergence and the beginning of tail resorption. Even more interesting than the effects of the treatments on mean body weights are the differences observed in the gonad lengths. At 53 days the mean gonad lengths of the various groups were: control, 0.772 mm.; thiouracil, 0.944 mm.; thyroxine, 0.602 mm. The difference between the thiouracil and control group is significant below the 2% level ("t" = 2.6450) and the differences be-

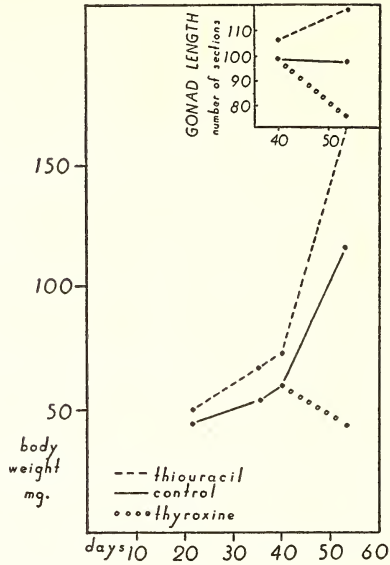


Fig. 1. Graphs showing the effects of thyroxine and 2-thiouracil on the mean body weights and gonad lengths of *R. pipiens* larvae.

tween the thyroxine and control groups are significant at the 1% level. This again shows the reversibility of the effect of thiouracil by thyroxine and indicates that the more rapid increase in the mean body weights of the thiouracil treated larvae is reflected in changes in the growth of the internal organs. It was also found that in 21 of the 25 pairs of gonads that were measured the left was longer than the right.

The results obtained from the *R. pipiens* larvae reared in aquaria were entirely negative. No significant differences were noted between animals treated with 0.0075% 2-thiouracil and the control larvae. The mean body weights of the 63 day samples were: controls, 193 mg.; thiouracil, 212 mg. The mean body weights of the metamorphosed animals were: controls, 824 mg.; thiouracil, 877 mg. These differences were not significant ($t = 0.427$ and 0.721 respectively). Even the decrease in the melanin pigment usually noticed to a variable extent in the thiouracil treated groups due to the inhibitory effect of this chemical on tyrosinase (2) was not observed, and it may be that the changes in pigment formation may serve as a crude measure of the anti-thyroid activity of a given dosage of thiouracil. However, the fact that a dosage of thiouracil insufficient to interfere with normal thyroid function does not alter larval growth also strengthens the contention that the effects of higher dosage levels are due to the action of this chemical on the thyroid gland.

The results of the individual measurements made on the right and left testes of mature male frogs were quite interesting. In 22 of the

24 pairs of *R. clamitans* testis weights the left was heavier than the right, while one showed no difference, and the remaining one showed the right to be slightly heavier. When the difference between the members of each pair is treated statistically the difference is found to be significant at the 1% level ("t" = 5.806). In the smaller sample of ten pairs of *R. catesbiana* testis weights the situation was quite similar and in only one pair was the right heavier than the left. These data suggest that difference observed in the lengths of the sectioned right and left gonads of the *R. clamitans* larvae (4) reflects a greater growth potential for the left side and also that the differences observed between the gonad lengths of the thiouracil and control larvae probably represent a real size difference.

Summary

Evidence is presented that 0.030% 2-thiouracil treatment of *R. pipiens* larvae results in a more rapid increase in body weight and gonad length than is observed in the controls. These results are reversible by thyroxine treatment. Treatment of *R. pipiens* larvae with a low dosage of thiouracil, (0.0075%), does not inhibit metamorphosis and has no significant effect on body weight. Thus, it seems likely that the effects of 0.030% thiouracil can be attributed to a hypofunction of the thyroid gland and that the thyroid secretion normally plays a role in controlling the growth of the pre-metamorphic Anuran larvae. Data are also presented that indicate that the left testis of *R. clamitans* and *R. catesbiana* frogs is usually larger than the right which strengthens the data obtained by measurements of microscopic sections of *R. clamitans* larvae in which a similar situation was observed.

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