Some Effects of 2-Thiouracil on *Rana clamitans* Larvae[1]

CHARLES H. STEINMETZ, Indiana University

Investigations concerning the thyroid gland in the larval forms of representative anurans have usually involved an analysis of experimentally produced hypofunction or hyperfunction with respect to retarded or accelerated metamorphosis. It has been shown that the thyroid glands of tadpoles begin to concentrate iodine very early in development (1). However, there is little evidence to indicate that the hormone is actually being secreted or, that if it is released in appreciable quantities, whether it bears any relation to premetamorphic ontogeny.

The purpose of these experiments was to investigate the effects of 2-thiouracil on non-metamorphosing *Rana clamitans* larvae to determine the time of the beginning of normal thyroid secretion and its role in larval ontogeny.

Materials and Methods

Thirty to 32 eggs obtained by induced ovulation (4) were placed in each of eight 8″ finger bowls filled with one liter of tap water and aerated continuously from the beginning of the experiments. The animals in two of these bowls were used for controls while each of the three levels of thiouracil employed was tested on the animals in two separate bowls of those remaining. The water was changed every other day and the thiouracil was added to the clean water to make concentrations of 0.015, 0.030, and 0.045%. The animals were permitted to feed on one gram of Pablum placed in the bowl 6-8 hours before changing the water. One of the control series and one of the two series tested with each level of thiouracil were weighed on the seventh day and every week on the same day throughout the course of the experiments. The other four series were not weighed as regularly. Body weights were determined by removing the animals one at a time to damp filter paper and transferring them by means of dry filter paper to the arm of a torsion balance and weighing to the nearest milligram. Animals studied histologically were fixed in Bouins' fluid, dehydrated in alcohol, embedded in paraffin, and 8 micron sections were stained with Harris' Haematoxylin and Eosin.

Results and Discussion

Effects on body weight—Days 1-21. There was no significant difference in the body weights in the groups of animals when the experiments began. Thiouracil treatment was begun on the ninth day and there was no effect detectable on the 14th; however, on the 21st day the average

[1] Contribution No. 458 from the Department of Zoology, Indiana University.

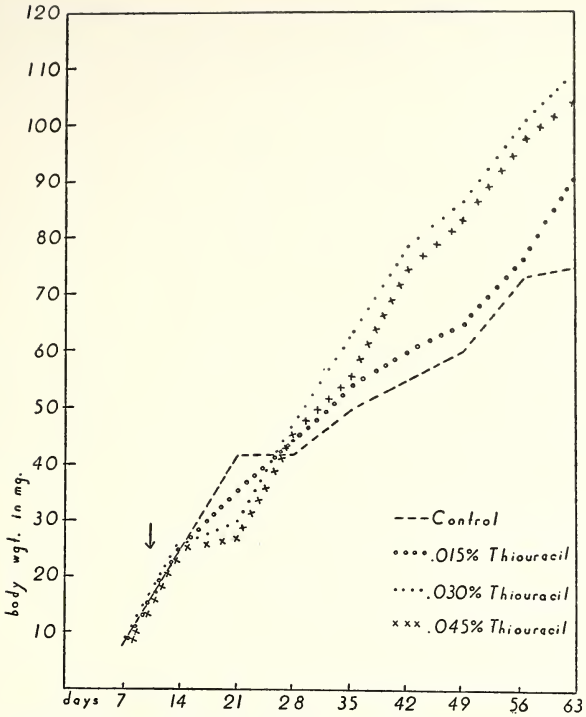

FIG. 1. Body weights of *R. clamitans* larvae weighed weekly from first to ninth week. Each point represents the average of weights of not less than 25 larvae. Arrow indicates beginning of thiouracil treatment.

body weights of all the thiouracil treated groups are significantly lower than those of the controls (Fig. 1). A sample of 10 of each of the series receiving the same treatments but not weighed on the 7th and 14th day, shows that this effect of decreased growth is not due to handling the animals. It is thought that this effect is due to an increase in the endogenous circulating thyroid hormone. Gordon et al (2) reported a notable decrease on the colloid content of thyroid glands of 20-30 mm. tadpoles after 10 days of treatment with 0.033% thiourea, and it has been noted previously in this laboratory that additional thyroid substance causes a decrease in the average body weight of treated larvae.

Effect on body weight—Days 28-63. The average body weights of the thiouracil treated animals, with the exception of the 0.015% group, are significantly greater than that of the control from the 35th day to the termination of the experiment. Growth is unquestionably greater in the animals treated with 0.030 and 0.045% thiouracil (Table I).

This conclusion is supported by the fact that the average body weights of samples of the series receiving the same treatments but

TABLE I. Average body weights of *Rana clamitans* larvae

Days		7	14	21	28	35	42	49	56	63
Treatment										
Control	(15)	7	24	42	42	48	53	58	71	72
				(3.21)	(0.88)	(1.33)	(1.66)	(0.85)	(0.47)	(1.48)
0.015%	(16)	7	24	35**	44	53	60	62	74	90
				(6.60)	(1.74)	(3.57)	(4.40)	(4.43)	(3.70)	(3.68)
0.030%	(17)	7	25	29**	46	62**	75**	85**	100**	109**
				(3.27)	(0.94)	(3.53)	(3.34)	(3.87)	(3.54)	(5.60)
0.045%	(18)	7	24	32**	45	56**	71**	81**	97**	104**
Control	(19)			43	48	55	54	49	55	
								(3.48)	(3.25)	
0.015%	(20)			33	46	54	61	59**	72**	
								(6.88)	(8.16)	
0.030%	(21)			33	51	64	75	76**	88**	
								(8.80)	(7.12)	
0.045%	(22)			31	54	63	76	81**	96**	
				Means of samples of ten weights						

**Differences are significant at the 1% level. Numbers in ( ) above the mean body weights of the experimental groups represent the "t" value of the difference from the controls.

not weighed in their entirety each week, showed the same significant difference for the treated larvae.

The possibility that crowding the animals is responsible for the effects observed is unlikely since the differences in the number of animals per group is slight and there are actually somewhat more in the treated groups toward the end of the experiments.

Thus the effect of 2-thiouracil with respect to growth varies with time. It would seem that thiouracil first results in a curtailment of hormone production and a release of stored colloid by the thyroid under the influence of the pituitary. This results in a temporary depression of growth. Then when the stored hormone is used up and no more can be produced by the thyroid the level of circulating hormone drops below normal and growth becomes excessive in the thiouracil treated larvae.

Effect on the gonads. The bodies of five animals from each of the four series sacrificed at 56 days were sectioned and the length of both the right and left gonads was determined by counting the number of sections in which a portion of the definitive gland appeared (Table II). The average gonad lengths of all the thiouracil treated groups are significantly greater than that of the control. With one exception, the right gonad is shorter than the left in the animals examined, but the same significant difference in length is noted for the right gonad alone in larvae treated with 0.030% and 0.045% thiouracil and in all the thiouracil treated groups for the left gonad alone. The length of both the right and left gonad show a high degree of positive correlation with

TABLE II. Measured and calculated mean gonad lengths of *Rana clamitans* larvae as determined from histological preparations. Animals were fixed on the 56th day after fertilization, after 47 days of treatment with the concentrations of thiouracil indicated.

Treatment	Average body weight in mg.	Average number of gonad sections				Calculated average gonad lengths	"t" for mean lengths
		Left	"t"	Right	"t"		
Control (19)	55.4	96	...	83	..	0.707 mm.	...
0.015% Thiouracil	68.6**	128**	8.83	79	1.16	0.787 mm.**	3.70
0.030% Thiouracil	73.0**	123**	3.39	99**	5.81	0.868 mm.**	9.87
0.045% Thiouracil	92.6**	139**	3.60	102**	3.71	0.931 mm.**	3.62

** Significant at 1% level.

the body weight giving correlation values of 0.5 and 0.8 respectively, both values being significant at the 1% level when tested by the "t" test (5).

Effect on the thyroid gland. The effect of thiouracil after 21 days of treatment is thought to be related to its effect on the thyroid gland resulting in a curtailment of hormone production. This is supported by an analysis of the thyroid glands made after 47 days of treatment. Gordon et al (2) noted a marked regression to an atrophic state at the end of two months of treatment with 0.033% thiourea on *R. pipiens* larvae. Stages in this regression are reflected by the glands at the various levels of thiouracil employed in these experiments. At the 0.015% level the glands are larger than the controls and the follicles contain greater amounts of colloid. The picture is about the same with some follicles showing signs of collapse at the 0.030% level. The glands of the animals treated with 0.045% thiouracil show few distinct follicles containing colloid and in some sections organization is almost completely lacking. Thus the effect of thiouracil on the thyroid gland seems to be roughly proportional to the dosage level which in turn seems directly related to the differences in the body weight and gonad lengths.

Effect on differentiation. Differentiation in general does not seem to have been affected by the thiouracil treatments. Presumably the developmental changes attributed to the thyroid are due to a higher level of secretion coupled with other changes in the physiology of the larvae at the time of metamorphosis. In these experiments the hypothyroid larvae are apparently simply larger while their organization and complexity is comparable to the control.

In speculating on the cause of these effects on body weights and gonads, some change in the pituitary function as a direct or indirect result of thiouracil treatment may offer a temporary working hypothesis.

Hoskins (3) noted hyperplasia of the hypophysis in thyroidectomized tadpoles and if such a phenomenon might be taken as an indication of increased secretion of growth and gonadotrophic factors, the results obtained would be clarified considerably. Further work on the role of the pituitary in these phenomena is already in progress.

Thus it might be concluded that the thyroid is actively secreting by the 35th day, if not before, as shown by the changes in the body weight. Further it would seem that this secretion is necessary either directly, or indirectly through its effect on other reactions, for maintenance of normal growth.

Summary

Effects on the body weights, gonad lengths, and histological appearance of the thyroid glands have been noted following treatment of *R. clamitans* larvae with 2-thiouracil. The relation of these effects to the beginning of normal secretion by the thyroid and its function in ontogeny have been discussed relative to the action of thiouracil.

Literature Cited

1. GORBMAN, A. and H. M. EVANS. 1941. Correlation of histological differentiation with beginning of function of developing thyroid gland of frog. Proc. Soc. Exp. Biol. & Med. **47**:103.
2. GORDON, A. S., E. GOLDSMITH, and H. CHARIPPER. 1945. The effects of thiourea on amphibian development. Growth **9**:19.
3. HOSKINS, E. R. and M. M. HOSKINS. 1919. Growth and development of amphibia as affected by thyroidectomy. J. Exp. Zool. **29**:1.
4. RUGH, R. 1934. Induced ovulation and artificial fertilization in the frog. Biol. Bull. **66**:22.
5. SNEDECOR, G. W. 1948. Statistical Methods. Iowa State Coll. Press.