EFFECTS OF THIOUREA UPON THE DEVELOPMENT
OF ANURAN LARVAE

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Up to the present, a considerable number of studies have been published on the role of the thyroid hormone in anuran development. The nature of this relationship, however, can not yet be stated to have been elucidated to satisfactory extent. Gordon, Goldsmith and Charipper (1943) reported that anuran metamorphosis was inhibited by thiourea treatment, and experiments on the same line have been performed by many subsequent investigators. With respect to such experiments, it is to be noticed that the workers have been principally concerned with the remarkable metamorphosis-inhibiting effect of antithyroidal substances, only a few dealing with the influence of the difference in experimental conditions, *viz.*, developmental stage of tadpoles, concentration of the drugs employed, duration of the treatment, etc. In order to disclose further informations about these problems, the following experiments were undertaken.

MATERIALS AND METHODS

Table 1. Materials and Procedures in the Present Experiments

Experiment	Material		Procedure			
	Species	Date of fertilization	Group	Initial age (days)	Initial total length (mm)	Concentration of thiourea (%)
1	Rhacophorus schlegelii	June 23	E	18	30	} 0.1 0.05 0.025 0.0125
			M	24	40	
			L	38	50	
2 { ser. 1	Rhacophorus schlegelii	April 28		46	50	0.025
		May 16		38	35	0.025
3	Rhacophorus schlegelii	May 13	E	13	21	0.025
			L	26	35	0.025
4	Rhacophorus schlegelii	May 14	E	16	20	} 0.025→0.033 (from 127th day)
			L	26	30	
5	Rhacophorus schlegelii	May 16	E	7	10	0.025
			la	32	32	0.1
			lb	32	32	0.0125
6	Rana japonica	April 21	E	13	9	0.025
			L	48	30	0.025
7	Rana japonica	March 11	E	26	9	0.025
			L	52	25	0.025
8 { ser. 1	Bufo vulgaris formosus	April 10		37	25	0.0125
		May 13	E	18	22	0.025
			L	27	26	0.025

Materials used were the tadpoles brought up from the eggs collected in the vicinity of Niigata City. Details of the materials employed and thiourea treatment are presented in Table 1. Tadpoles were reared on boiled spinach. Solutions constituting experimental environment was changed every 2~4 days. Emergence of the fore-limbs was resorted to as the criterion of metamorphoses. Age of the tadpoles was enumerated by days after fertilization.

RESULTS

Exp. 1. As shown in Table 2, the development of E was generally retarded after 53 days, which was particularly remarkable in the hind-limbs. The animals of

Table 2. Effects of Thiourea on the Growth of Anuran Larvae

Exp.	Age (days)	Group	Total length*	Body length*	Body breadth*	Tail-fin height*	Hind-limb length*
1	53	E { 0.025 %	52.9±2.7	17.2±0.8	11.5±1.3	12.2±1.0	2.9±0.8
			49.1±2.9	16.8±0.7	10.2±1.1	12.4±0.7	2.6±0.9
			43.7±3.1	15.3±0.8	8.9±1.4	9.8±0.9	1.9±0.5
		M { 0.025	53.2±2.5	18.8±0.6	11.5±0.9	11.9±0.9	11.3±3.8
			52.2±2.8	18.4±0.5	11.3±1.0	11.7±0.6	11.0±4.0
			51.5±2.7	17.4±0.9	10.9±1.3	10.8±0.7	10.0±3.5
	56	E	57.3±3.3	19.9±1.0	12.7±1.3	13.5±1.1	2.5±0.4
		L	58.3±3.4	19.6±0.9	12.9±1.0	13.0±1.0	6.1±2.3
	77	Control		17.1±0.6			22.9±1.2
		E	63.1±3.1	21.5±1.1	14.3±1.3	15.6±1.0	4.3±2.4
4	100	L	64.8±4.2	21.7±1.3	14.6±1.9	15.0±1.4	10.3±4.7
		E	59.7±3.0	21.1±1.1	13.7±1.0	14.8±1.2	4.3±2.2
	78	L	59.1±2.8	21.0±1.2	14.2±1.2	14.9±1.3	10.1±4.6
		E	38.6±2.9	13.4±1.0	8.6±0.7	6.9±0.7	2.6±0.5
6	99	L	42.8±2.5	14.1±0.7	9.8±0.6	7.5±0.7	6.4±0.7
		Control		15.6±0.9			22.8±1.9
	99	E	43.1±3.1	14.7±1.1	9.3±1.1	7.9±1.0	4.3±0.9
		L	46.1±2.8	15.2±0.8	10.7±1.0	8.7±1.2	10.3±4.5
8	32	E	26.9±0.5	8.9±0.7	4.8±0.3	4.3±0.3	5.9±0.8
		L	26.1±1.1	9.0±0.5	4.7±0.3	4.5±0.4	7.3±1.3
		Control	26.3±0.9	8.9±0.6	4.9±0.2	4.5±0.4	7.5±0.8

* measured in mm

all the groups of L and 0.0125% groups of E and M already metamorphosed. As shown in Figure 1, metamorphosis was slightly accelerated in L than in the controls regardless of thiourea concentration. The animals of 0.0125% groups of E and M metamorphosed 3~4 days later than the controls. In the other groups, metamorphosis was inhibited. Eight days after the metamorphosis of the controls, each non-metamorphosed group was divided into two lots. In one the tadpoles were transferred to tap water, and in the other, the tadpoles were continuously kept in thiourea solutions. In the former, the stronger the influence of preceding treatment, the more significantly retarded was the advance of metamorphic process. It appeared, however, that the influence of the developmental stage at which thiourea treatment was initiated was rather more pronounced than that of thiourea concentration. While, in the latter, none of the tadpoles metamorphosed until the end of the experiment.

Exp. 2. As shown in Figure 2, the total length as well as the body length of thiourea-treated groups was smaller than those of the controls even at the end of 1 or 2 days after the initiation of thiourea treatment. The development of

Fig. 1.

Fig. 2.

the hind-limbs of thiourea-treated groups, however, was at the same rate or rather accelerated as compared with that of the controls for several days, and was markedly inhibited thereafter.

Exp. 3. Metamorphosis in the controls occurred on the 46th~54th days. As shown in Table 2, the total length, the body breadth and the hind-limb length in L were larger respectively than those in E. Tail-fin height in L, however, was less than that in E. A relatively wide-scaled variation was observed in the hind-limb length of thiourea-treated animals. On the 59th and 71st days respectively, one animal in L completed metamorphosis, though none of the animals metamorphosed in E.

Exp. 4. Tadpoles were kept at room temperature until the middle of October and at 18°C thereafter. Metamorphosis of the controls occurred on the 49th~56th days. As shown in Table 2, no difference was observable between E and L except for the hind-limb length on the 100th day. Thirty days thereafter, the difference in the hind-limb became less pronounced. Metamorphosis of thiourea-treated groups was remarkably inhibited. As shown in Figure 3, however, some

Fig. 3.

Fig. 4.

of the animals of both E and L groups metamorphosed. When they were transferred to more concentrated solution, metamorphic change was suppressed much more pronouncedly.

Exp. 5. As shown in Figure 4, growth of thiourea-treated animals was of the order $Lb > E > La$ on the 104th day (51 days after metamorphosis of the controls). On the 164th day, however, growth rate in La increased more than that in E, and the difference between the two groups slightly diminished.

Exp. 6. Retardation of growth in E was ascertained in one week after the beginning of thiourea treatment. As shown in Table 2, growth in L was predominating that in E. On the 99th day, variation in the hind-limb length was considerably great in L. The controls metamorphosed on the 64th~85th days. While, in thiourea-treated groups, progress of metamorphic change was extremely inhibited.

Exp. 7. As shown in Figure 4, growth in L was more advanced than that in E on the 105th day (several days after metamorphosis of the controls). While, on the 146th day, there was little or no difference between the two groups. On the 246th day, the total length, the body breadth and the hind-limb length of E were predominating those of L.

Exp. 8. In series 1, as shown in Figure 5, the beginning of metamorphosis in thiourea-treated animals was little delayed as compared with that of the controls.

Fig. 5.

Fig. 6.

In series 2, as shown in Figure 6, metamorphic changes in L and the controls began almost simultaneously. In L, however, a definite retardation of tail-resorption process was observed, and in some of the individuals tail resorption did not occur at all for half a month or more after the emergence of the fore-limbs. Beginning of metamorphic change was considerably retarded in E. Body size in each group on the 32nd day is shown in Table 2. Almost no difference was observable among those groups except for the hind-limb length.

DISCUSSION

Steinmetz (1954) reported that hypothyroid condition was observed in *Rana clamitans* tadpoles which were kept in a 0.03% thiourea solution, and that thiourea caused an increase in the rate of growth without affecting differentiation. Thomas (1947) observed remarkable inhibition of growth in *Rana pipiens* tadpoles which were treated with 0.05% thiourea. In the present study, the growth of the tadpoles immersed in thiourea solutions for 2~3 months was inhibited, depending on the stage of initiation of the treatment and also on the concentration of thiourea. The results obtained by Steinmetz are likely to be attributed to species' specificity.

It is widely known that amphibian metamorphosis is inhibited by antithyroidal substances (Gordon, Goldsmith and Charipper, 1943, 1945; Hughes and Astwood, 1944; Gasche, 1946; Thomas, 1947; Lynn, 1948; Schindler, Schär and Staehelin, 1948; Delsol, 1952; Hanaoka, 1954; etc.). Gasche (1946) reported inhibition of metamorphosis in *Xenopus laevis* tadpoles which were kept in a 0.04% thiourea solution from the early stage of larval life. Hanaoka (1954) likewise demonstrated that the large tadpoles of *Rana temporaria* which were treated with 0.025% thiourea failed to develop beyond the premetamorphic stage. Gordon, Goldsmith and Charipper (1945) reported that the large tadpoles of *Rana pipiens* immersed in 0.033% thiourea continued to advance at metamorphosis but at significantly slower rate than that exhibited by the controls. In the present study, the larvae of *Rhacophorus schlegelii* immersed in 0.0125% thiourea at the middle larval stage metamorphosed several days later than the controls. In a 0.025% solution, metamorphosis of the tadpoles was remarkably inhibited, but some of the tadpoles continued to advance gradually at metamorphosis for longer periods of time. When a little more concentrated solution was used, advance at metamorphosis was almost perfectly inhibited. It therefore appears that metamorphosis of the tadpoles immersed in a less concentrated solution of thiourea is due to possible continuance of production of a small quantity of the thyroid hormone rather than the release of the hormone stored in the gland before the initiation of the treatment. Unlike *Rhacophorus schlegelii* and *Rana japonica*, larval development of *Bufo vulgaris formosus* was little affected by a 0.025% thiourea solution, though earlier initiation of the treatment brought about a considerable inhibition of the growth and metamorphosis of the animals. It may be considered that the degree of metamorphosis-inhibiting effect of thiourea is dependent upon the stage of the initial treatment, concentration of thiourea, difference in species, and so on.

As Gordon, Goldsmith and Charipper (1945) and Gasche (1946) pointed out, cessation of thiourea treatment brought about a resumptive advance in the metamorphosis of tadpoles. The rate of resumed development in tap water was concerned with the intensity of preceding thiourea treatment.

Roth (1947) has reported that thiourea treatment brought about accelerated metamorphosis in *Rana temporaria* larvae. At present the nature of the results obtained by Roth is unknown. In the present study, temporary increase in the rate of the hind-limb development was observed in greater or lesser degrees in thiourea-treated tadpoles, and the progress of metamorphic changes in the full-

grown tadpoles was somewhat accelerated. As has been described in the previous paper (Iwasawa, 1952), intact larvae of *Triturus pyrrhogaster*, that failed to advance at metamorphosis on account of some unknown factors, metamorphosed as the result of thiourea treatment. It is conceivable that the facts described above indicate a passing hyperthyroidism-like condition caused by thiourea treatment. The investigation on the nature of such phenomena would warrant further research.

SUMMARY

Tadpoles of *Rhacophorus schlegelii*, *Rana japonica* and *Bufo vulgaris formosus* were treated with thiourea of various concentrations, and effects of thiourea on the growth and metamorphosis were examined.

1. A passing hyperthyroidism-like condition was observed directly following the initiation of thiourea treatment.

2. When the treatment was initiated at earlier tadpole stage and much concentrated solution of thiourea was used, the growth of tadpoles was much more significantly inhibited. When tadpoles were treated for longer periods of time, however, reversed was the case.

3. Metamorphic change of the animals was inhibited when treated with thiourea from the early tadpole stage. Among those animals, however, considerable individual variations were observed, and in less concentrated solutions, some of the treated animals continued to advance at metamorphosis.

4. It appears likely that a small quantity of the thyroid hormone might be produced in spite of thiourea treatment.

5. The treated animals metamorphosed shortly after the cessation of thiourea treatment. The rate of resumed development, however, was learned to be dependent on the magnitude of the preceding treatment.

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