The Effect of Thyroxine on the Metamorphosis in Hypophysectomized and in Thyroidectomized Frog Tadpoles (*Rana ornativentris*)[1)]

By

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(*With 4 Text-figures and 1 Table*)

It is already established that thyroid hormone induces metamorphic events in both the hypophysectomzied and the thyroidectomized frog tadpoles. However, the mode of action of thyroid hormone on the metamorphosis still remains a question. Allen (1932) and Etkin (1935) have described that in order to accomplish metamorphic change each tissue has a different total thyroxine requirement which is satisfied either by higher concentration acting in a shorter period or by a lower one over a long period of time. On the other hand, Kollros (1961) has reported that low concentration of thyroxine induces the tadpoles to a particular point in metamorphosis and that they remain at that point as long as the concentration is not raised. However, these different results can not be directly compared, because both Allen and Etkin have used the thyroidectomized tadpoles in their experiments whereas Kollros has used the hypophysectomized tadpoles in his experiments. The present experiment was designed to compare the metamorphic rate induced by thyroxine treatment in the hypophysectomized and the thyroidectomized tadpoles of the frog, *Rana ornativentris*.

Material and Method: The tadpoles used in the present experiments were obtained from one batch of eggs of *Rana ornativentris* which were collected at Mt. Akagi in Gunma Prefecture. Thyroidectomy was performed under anesthesia with MS 222 at stage 20 or 21 by a modification of Allen's method (1918). A vertical cut was made through the middle of the oral suckers with an edged needle under a binocular dissecting microscope and the endodermal tissues of the prospective thyroid gland were taken out from the floor of the pharynx by using a hair loop and a glass needle. Hypophysectomzied tadpoles were obtained by extirpating hypophysial anlage at tailbud stage by the usual method of Allen (1916) and Smith (1916). These tadpoles were raised at room temperature and fed boiled spinach or Chinese cabbage. All the operated tadpoles were not used until 12 weeks after normal tadpoles metamorphosed. Forty-four thyroidectomized tadpoles and

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62 hypophysectomized tadpoles were obtained and they were divided into 6 groups as follows:

I-A. Twenty-one hypophysectomized tadpoles. No thyroxine treatment.

I-B. Twenty-one hypophysectomized tadpoles treated with 2×10^{-11} moles thyroxine solution for 22 days, and then concentration was raised to 5×10^{-11} moles.

I-C. Eighteen hypophysectomized tadpoles treated with 2×10^{-10} moles thyroxine solution.

II-A. Eight thyroidectomized tadpoles. No thyroxine treatment.

II-B. Seventeen thyroidectomized tadpoles treated with 2×10^{-11} moles thyroxine solution for 22 days, and then the concentration was raised to 5×10^{-11} moles.

II-C. Nineteen thyroidectomized tadpoles treated with 2×10^{-10} moles thyroxine solution.

The thyroxine solution was changed every two days. In all the experimental animals, the total length, hind limb length and metamorphic stage according to Taylor and Kollros (1946) were observed every 8 days for 32 days. Ten of the hypophysectomized tadpoles, which were treated with 2×10^{-10}M thyroxine for 32 days (Group I-C), were transferred to 5×10^{-10} M thyroxine and the other eleven remained in the same concentration.

Results

As shown in Figures 1 and 2, both the hypophysectomized and thyroidectomized tadpoles advanced from stage IX to stage XIII 12 weeks after operation. At the end of the experiments, distribution of the metamorphic stages among hypophysectomized tadpoles was from stage XI to XIII, whereas the thyroidectomized tadpoles showed a little more advancement and reached from stage XIII to XIV. These results have been confirmed by the observation made on one year old hypophysectomized and thyroidectomized tadpoles.

In the lower concentration (2×10^{-11} M), the metamorphosis was not accelerated for 22 days in both the hypophysectomized and the thyroidectomized tadpoles (Figs. 1 and 2). The hind limb length did not increase as compared with that of the control animals (Figs. 3 and 4). Twenty-two days after the thyroxine treatment was started the concentration of thyroxine was raised to 5×10^{-11} M. At 10 days of treatment both the hypophysectomized and thyroidectomized tadpoles showed a little advancement of metamorphosis and an increase in the hind limb length (Figs. 1, 2, 3 and 4).

The higher thyroxine solution (2×10^{-10}M) induced rapid metamorphic changes in both the hypophysectomized and the thyroidectomized tadpoles at nearly the same rate. As shown in Figures 3 and 4, growth of the hind limbs responded well to thyroxine and the tadpoles advanced from stage XVII to XIX at 32 days of treatment (Figs. 1 and 2). Some of the hypophysectomized and the thyroidectomized tadpoles already advanced to stage XIX in 16 days of treatment and stopped at this stage thereafter. When the thyroxine concentration was raised to 5×10^{-10} M (Group I-C) the forelimbs emerged in the hypophysectomized tadpoles, whereas if the concentration was not raised the tadpoles remained at the same stage.

The total length of the tadpoles increased in all groups at the end of the experiment (Table 1). However, the difference in the total length between the thyroxine-treated and the untreated tadpoles was not significant.

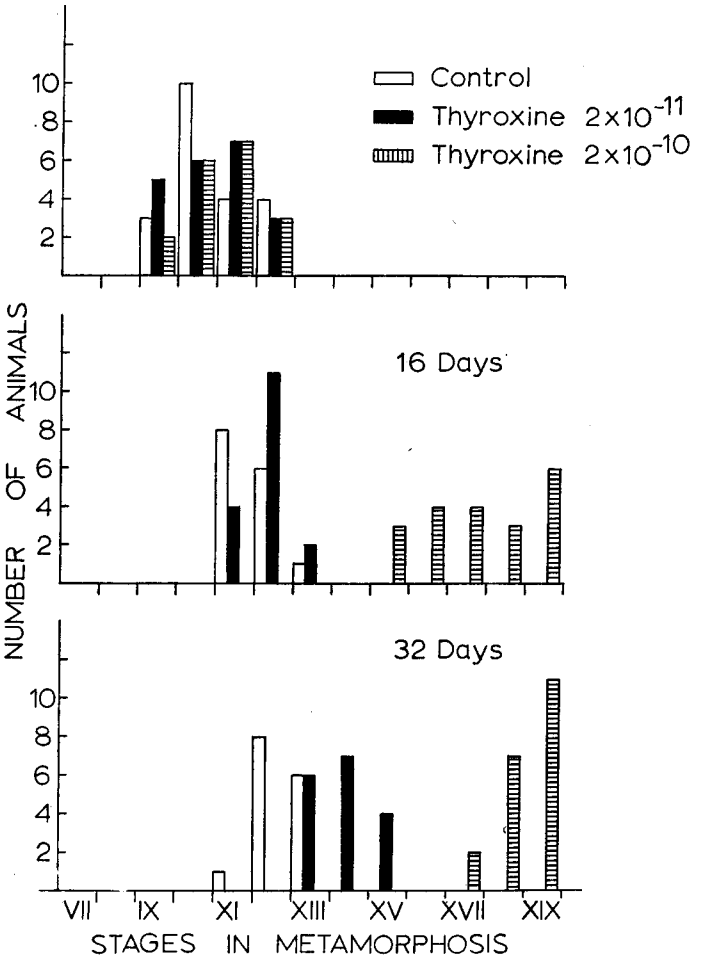

Figure 1. Distribution of metamorphic stage of hypophysectomzied tadpoles treated with thyroxine.

Discussion

It has been demonstrated that the metamorphosis of the hypophysectomized tadpoles is arrested usually at stage VII in *Rana pipiens* (Kollros and McMurray, 1956) and in *Hyla regilla* (Eakin and Bush, 1957). In the present experiment, however, the hypophysectomized and the thyroidectomized tadpoles advanced to stages XIII and XIV, respectively. Advancement to stage XIII in hypophysectomized tadpoles and to stage XIV in thyroidectomized tadpoles has also been observed in

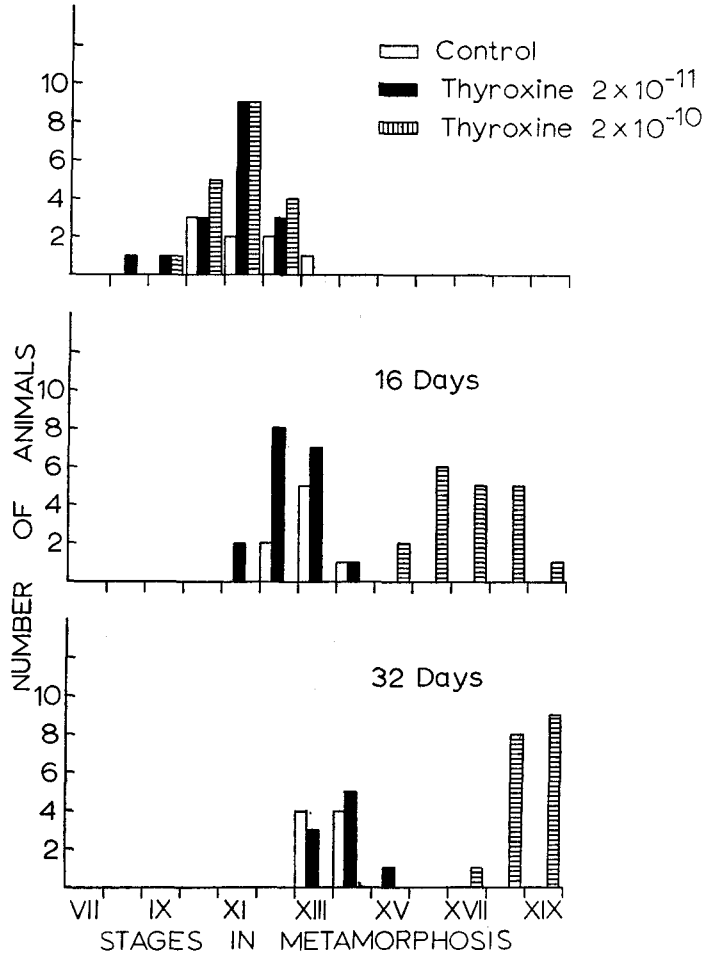

Figure 2. Distribution of metamorphic stage of thyrodiectomized tadpoles treated with thyroxine.

one year old tadpoles of *Rana japonica* (Hanaoka, unpublished). The advancement of the metamorphic stage of the tadpole after hypophysectomy or thyroidectomy was apparently different in different species. Furthermore, the fact that both the hypophysectomized and the thyroidectomized tadpoles advanced to about the same metamorphic stage seems to show that the tadpole thyroid gland becomes practically nonfunctional following hypophysectomy.

It was found that the minimum concentration of thyroxine required for any metamorphic changes was approximately 5×10^{-11} M (about 0.04 μg/litter) in

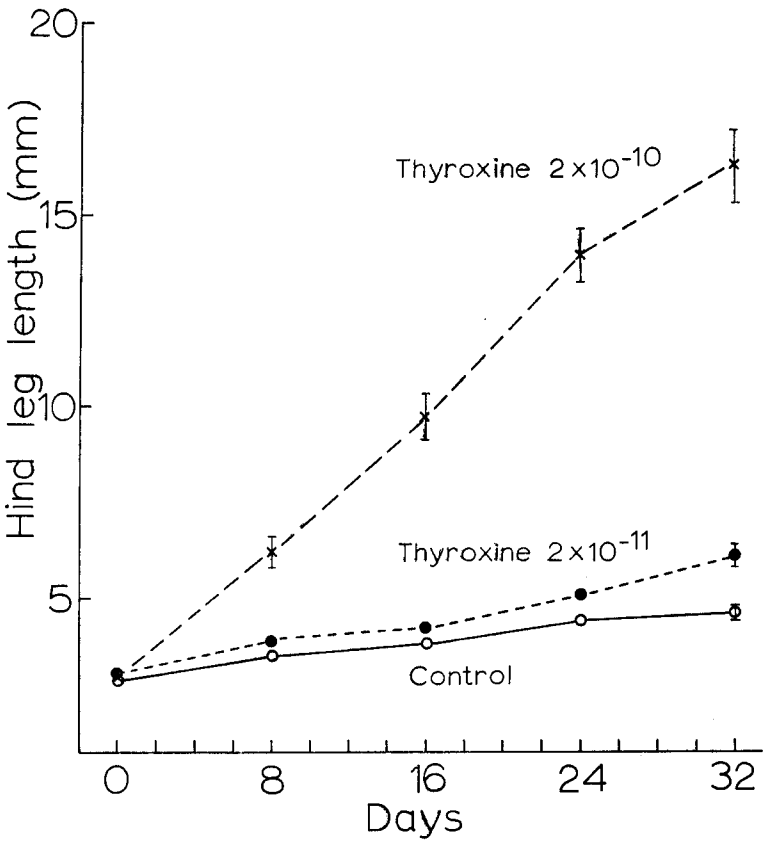

Figure 3. Growth of hind limb in the hypophysectomized tadpoles treated with thyroxine.

Table 1. Effect of thyroxine on the total length of tadpoles

	Group	No. of animals	Doses of thyroxine	Mean total length (mm)	
				Initial	Final
Hypophysectomized tadopoles	I-A	21	None	48.3±0.9*	54.0±1.1
	I-B	21	2×10^{-11}M	48.4±0.9	52.6±0.8
	I-C	18	2×10^{-10}M	50.2±1.2	53.1±2.0
Thyroidectomized tadpoles	II-A	8	None	53.3±1.1	62.0±1.1
	II-B	17	2×10^{-11}M	52.8±0.6	61.9±1.2
	II-C	19	2×10^{-10}M	53.4±1.0	61.5±0.9

* Mean ± standard error.

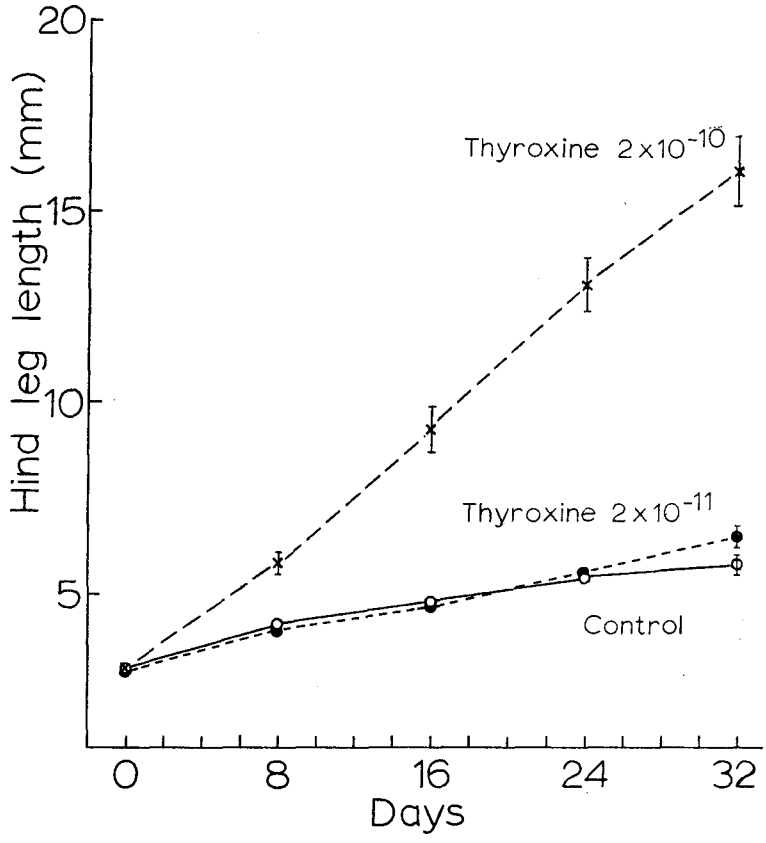

Figure 4. Growth of hind limb in the thyroidectomized tadpoles treated with thyroxine.

Rana ornativentris. This concentration was much lower than the value of about 1 μg/liter which Witschi (1956) and Etkin (1964) noted as a slightly effective concentration, but higher than the value of 0.002 μg/liter which Kollros (1961) demonstrated as a real minimum effective concentration in *Rana pipines*. With 2×10^{-10}M thyroxine (about 0.16 μg/liter) the tadpoles advanced to stage from XVII to XIX in both the hypophysectomized and the thyroidectomized tadpoles in 32 days after treatment. Some of the tadpoles already reached the stage XIX at 16 days after thyroxine treatment and remained unchanged for 16 days at this stage, whereas the intact tadpoles usually stayed at this stage for only 2 or 3 days during the normal metamorphosis. The emergence of the forelimb required up to 5×10^{-10}M of thyroxine (about 0.4 μg/liter). The concentration required for the beginning of metamorphic climax was about the same value as in the experiments of *Rana pipiens* (Kollros, 1961).

Using the thyroidectomized tadpoles, Allen (1932) and Etkin (1935) reported that the low concentration of thyroxine, which induces the early metamorphic events, is capable of leading the tadpoles to the late metamorphic changes if it acts long enough. They have described that true thresholds to thyroxine does not exist in the tissue of the tadpole. On the other hand, Kollros (1961) has demonstrated in the hypophysectomized tadpoles that late metamorphic change such as the emergence of the forelimb and the resorption of the tail is not induced by the lower concentration, which causes differentiation of the hind limb. The lower concentration of thyroxine only induces the tadpole to a certain point of metamorphosis in spite of treatment continued for more than one month. In such a stable tadpole the metamorphosis progresses only when the concentraion is raised. Therefore, with a suitable concentration thyroxine can separate temporarily one metamorphic event from the following ones. The question arises whether thyroxine leads the metamorphic change at a different rate between the hypophysectomized and the thyroidectomized tadpole. Little work has been done on the difference between the effect of thyroxine on the hypophysectomzied tadpoles and on the thyroidectomized ones. A possibility exists that somatotropin or other hormones from the pituitary act complementary with thyroxine on the metamorphic events. There is evidence that thyroid hormone and somatotropin act synergistically in promoting normal skeletal growth (Asling *et al.*, 1954). Tonoue and others (1963) reported that thyroid stimulating hormone acts directly on peripheral tissue to increase thyroxine uptake.

However, contrary to expectation, it was clearly demonstrated from the present experiment that thyroxine induces the metamorphic events in both the hypophysectomized and the thyroidectomized tadpoles at nearly the same rate. This fact indicates that metamorphosis is mostly dependent on the thyroid hormone and that hypophysial hormones do not affect the rate of metamorphosis induced by thyroxine. With 2×10^{-10}M thyroxine treatment on the thyroidectomized tadpoles, an apparent stasis of metamorphosis was observed just before the forelimb emergence. Kollros (1961) has reported that many such stable tadpoles in different stages are induced by thyroxine treatment in hypophysectomized tadpoles. These observations support the statement that thresholds of thyroxine for metamorphic events do exists in both the thyroidectomized and the hypophysectomized tadpoles.

Summary

The hypophysectomized *Rana ornativentris* tadpoles were shown to advance to stage XIII, the the thyroidectomized ones to stage XIV. The rate of metamorphic changes induced by thyroxine was almost the same in both groups. It was noted that the threshold of thyroxine for each metamorphic event is not affected by hypophysial hormones.

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