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THE ENVIRONMENTAL EFFECT ON THIOUREA-INDUCED SEX TRANSFORMATION IN *RANA CATESBEIANA* TADPOLES

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ABSTRACT

Tadpoles of undifferentiated sex race of *Rana catesbeiana* were subjected to thiourea treatment. The aquaria were placed by the windows, thus exposing to sunlight. After 9 months of treatment, gonads were sexed macroscopically and microscopically. Sex distribution toward male type in both control and treated animals was found; the treated tadpoles were more affected. The result was different from that of a previous similar experiment in which only the experimentals showed an altered sex distribution. It was assumed that thiourea and high temperature synergistically accelerated sex differentiation, thus resulting in a preponderance of male population.

The trend of sex development in undifferentiated sex race of frogs is from female to male type (1), the rate of which is controlled by intrinsic physiological and extrinsic environmental factors, resulting in ultimately a balanced 1:1 sex ratio. However, this equilibrium can be overthrown by a number of agents. A previous study demonstrated the action of thiourea on acceleration of sex transformation in undifferentiated sex race of *Rana catesbeiana* tadpoles (2). The present paper reports a similar experiment, but the environmental factor other than thiourea was altered.

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MATERIALS AND METHODS

Fertilized eggs were obtained in April, 1965 from induced breeding of *Rana catesbeiana* of undifferentiated sex race. Two months after fertilization, 200 tadpoles, at the stage when their hindlimb buds were just beginning to appear with an average total length of 20 mm, were subjected to the treatment of 0.033% thiourea in dechlorinated tap water. Two hundred control tadpoles were reared in dechlorinated tap water only. The rearing medium was renewed daily except Sundays. All tadpoles were fed with thawed leaves of water convolvulus *ad libitum*. Room temperature was averaged at 22.9 ± 1.3 C.

Five months after fertilization, some

newly metamorphosed froglets in the control group were sacrificed for sex examination. Six months later, control frogs and over-winter tadpoles and thiourea-treated tadpoles were all killed for gonadal inspection. The representative gonads of various types in each group were fixed in Bouins' fluid, embedded in paraffin, cut in serial sections of 10 *u* and 7 *u* respectively for ovaries and testes and stained with haematoxylin and eosin. Individuals were sexed according to both macroscopic and microscopic examination.

Thyroid glands of the 2 groups of tadpoles were prepared histologically to follow up the goitrogenic effect.

RESULTS

Metamorphosis of thiourea-treated tadpoles was inhibited as usual at stage II or III which was confirmed by the increase of epithelial height and decrease of the amount of colloid in the thyroid gland (*Figs. 2* and *3*).

The control and thiourea-treated gonads were respectively classified into 3 types according to their size and shape as indicated in *Fig. 1*. Ovaries were large bodies and testes small while intersexes occupied a wide range in between as described previously (2).

Histological examination found the testis composed of spermatogonia and developing seminiferous tubules (*Fig. 4*). Two or 3 germ cells might be enveloped together by a few small and darkly stained cells of connective tissue origin (*Fig. 5*). The ovary was full of large, healthy auxocytes in the growing stage; each was incased with a thin layer of follicular cells (*Fig. 6*). The egg cells contained large, vesicular and lightly stained nuclei. The gonad of intersex appeared as a composite structure of testicular tissue and degererating oocytes, the latter being interposed mostly in the poterior part of the gonad (*Fig. 7*). The 2 elements might vary in proportion according to the degree of transformation.

Macroscopically and microscopically, the 3 types of control gonads were not different respectively from those of the thiourea-treated tadpoles.

As reported previously, *Rana catesbeiana* in Taiwan belongs to undifferentiated sex race in which tadpoles are exclusively female (2). However, the results of the present experiment indicated an early sex differentiation in control frogs with a preponderance of male individuals while control tadpoles showed 4 males, 6 hermaphrodites and 67 females (TABLE I). Therefore, the control animals exhibited a precocious development of gonads when compared with the controls of the previous study (2).

The experimentals consisted of 27 females, 30 intersexes and 38 males (TABLE I), the ratio of which was also different from that of the previous study.

TABLE I

Sex distribution of control and thiourea-treated tadpoles

	Month after fertilization	Stage	Sex ♀	Sex ⚥	Sex ♂	Total
Control						106
Froglets	5		5	0	3	
Frogs	11		3	0	18	
Over-winter tadpoles	11	III to XVI	67	6	4	
Experimental tadpoles	11	II & III	27	30	38	95

DISCUSSION

TABLE II shows the difference in sex distribution between the 2 experiments. It was evident that both control and thiourea-treated animals in the present experiment accelerated their sex differentiation toward male type.

TABLE II

Comparison of sex distribution in the 2 experiments

	Controls						Thiourea-treated tadpoles		
	Frogs			Tadpoles					
Previous report	♀	⚥	♂	♀	⚥	♂	♀	⚥	♂
No.	7	3	0(12)*	22	0	0(11)	50	5	17(7)
%	70	30	0	100	0	0	69.5	6.9	23.6
Present report									
No.	8	0	21(11)	67	6	4(11)	27	30	38(11)
%	27.6	0	72.4	87.0	7.8	5.2	28.4	31.6	40.0

*Figures in parenthesis denote age of tadpoles or frogs in month.

The tadpoles used in the 2 experiments were of the same species; but those of the previous study were from natural ovulation whereas tadpoles of this experiment from induced breeding. Both pairs of parent frogs came from the same source, a bullfrog farm in Taichung. Thus it was assumed that tadpoles of the 2 experiments belonged to the same undifferentiated sex race

Witschi reported that "not fully ripened eggs accelerated the onset of male differentiation" (1). In the present experiment eggs were carefully observed before artificial fertilization took place in the laboratory. Thus there was hardly any chance for unripened or overripe eggs to be fertilized.

Thiourea concentration in the 2 experiments was the same. The treatment lasted for 5 months in the previous study whereas the same treatment took 9 months in this experiment. It could have been the 4 more months which caused an excess of males in this experiment. However, the controls showed the same trend. Therefore there must be other factor(s) responsible for the difference in sex distribution.

The 2 experiments were started in the same season of the year; eggs were fertilized in April of 1963 and 1965 respectively. The monthly room temperature of the 2 years were comparable (TABLE III).

TABLE III

Monthly temperature, C, 1963-1966

	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mean+SE
1963	22.3	27.0	26.6	27.9	28.7	27.8	23.1	21.5	16.8	15.7('64)	15.1('64)	22.95±1.54
1965	21.0	24.0	25.9	28.3	28.5	26.0	24.1	21.9	17.5	16.7('66)	17.6('66)	22.86±1.28

However, the aquaria of the present experiment were placed by the windows, thus exposing to sunlight. In the previous experiment, they were not sunshined. Hence, the ambient water of the tadpoles in this experiment was warmer than that of the previous one, especially in the summer season between July and September when most of the tadpoles metamorphosed.

Witschi stated that the rate of sex transformation in local races of frogs in Europe is controlled by a temperature factor of the climatic complex (3) and demonstrated sex reversal in female tadpoles of *Rana sylvatica* following the application of high temperature (4). Therefore it might have been the warm environment which induced the tadpoles of undifferentiated sex race in this experiment to speed up their sex differentiation, resulting in more males in both control and experimental groups. The result thus indicated a synergistic action of high temperature and thiourea on sex development. This, however, should be confirmed by experiment with controlled temperature.

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LEGEND OF FIGURES

Fig. 1. Gonads with kidneys of the undifferentiated sex race of *Rana catesbeiana.* × 60. Left, ovaries; middle, intersex; right, testes.

Fig. 2. Cross section of the thyroid gland of a normal *Rana catesbeiana* tadpole. × 300.

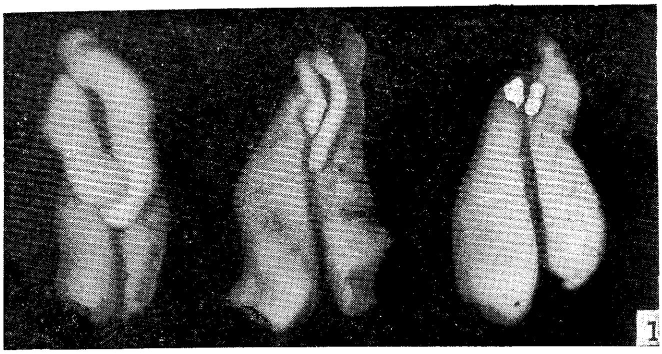
Fig. 3. Cross section of the thyroid gland of a thiourea-treated tadpole of *Rana catesbeiana.* × 300.

Fig. 4. Sagittal section of the testis of the young frog of *Rana catesbeiana.* × 150.

Fig. 5. An enlarged portion of *Fig. 4.* × 300.

Fig. 6. A portion of the sagittal section of the ovary of a *Rana catesbeiana* tadpole. × 150.

Fig. 7. Sagittal section of the transforming gonad of an intersex of *Rana catesbeiana* tadpole. × 150.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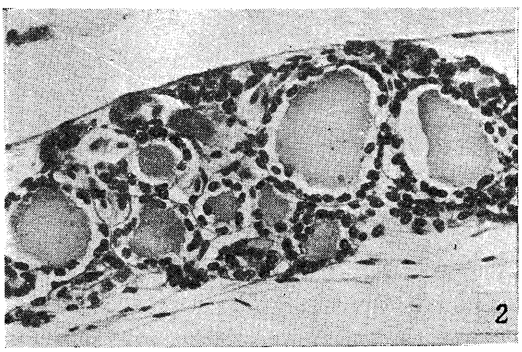
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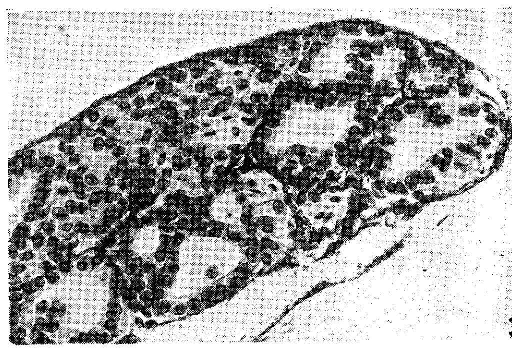
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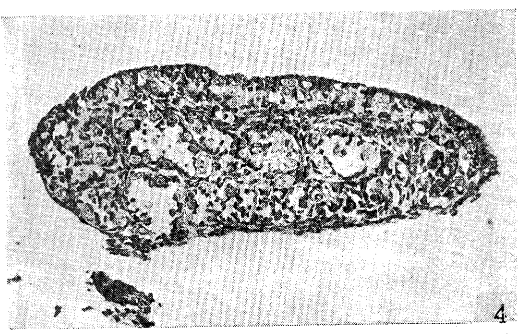
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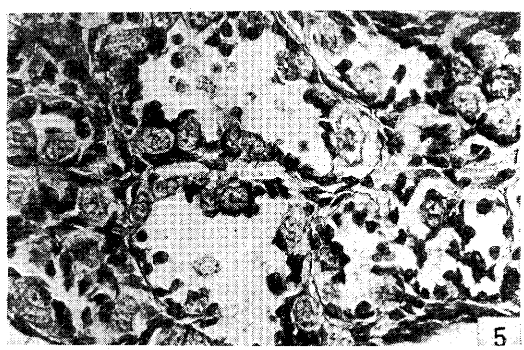
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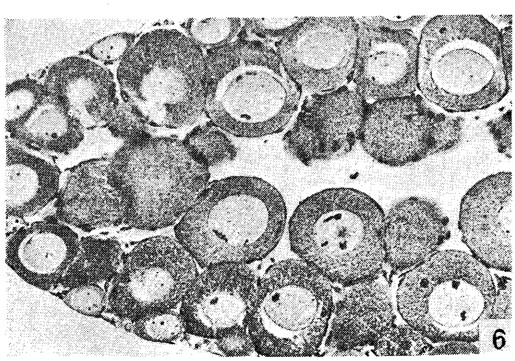
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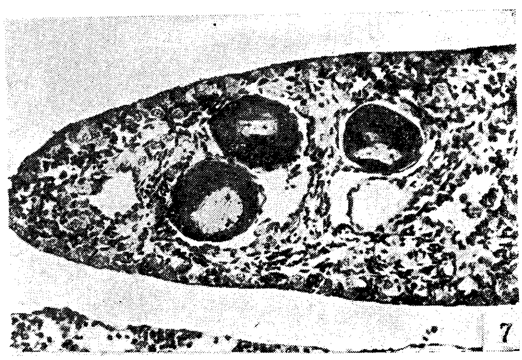
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