SEX REVERSAL IN FEMALE TADPOLES INDUCED BY THE TREATMENT WITH METHYLMERCAPTOIMIDAZOLE

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Since Gordon et al. (1943) reported that amphibian metamorphosis is effectively inhibited by thiourea treatment, a considerable number of studies have been made by many investigators to examine the effects of antithyroidal substances on amphibian larvae. Most of them, however, dealt with only the inhibition of metamorphosis or the dipigmentation, and no description on the gonads of the treated larvae was found out. The author previously reported on the transformation of ovaries to testes (Iwasawa, 1955a, b, 1958a) and on the precocious germ cell differentiation (Iwasawa, 1957a) in thiourea-treated larvae. The drugs used by foreign investigators were mostly thiouracil, propylthiouracil or phenyl-thiourea, and occasionally, thiourea. Consequently there remains some reason to think that the above mentioned changes observed in the gonads of thiourea-treated larvae were not the common effect of antithyroidal substances, but the specific effect of thiourea. Of course there are other important points to be considered as the causes of this difference, that is, type of sex differentiation, correlation among the endocrine glands, responsibilities of the endocrine organs for antithyroidal substances, and so on. These matters will be discussed in the ensuing papers. 1-methyl-2-mercaptoimidazole is a newly discovered antithyroidal substance, and its stronger antithyroidal effect and lesser by-effects in mammals are already recognized. Therefore, in the present paper, the effects of this substance upon the gonadal development of Rhacophorus larvae were dealt with.

The author wishes to thank Prof. I. Motomura of the Tohoku University for his valuable suggestion and encouragement.

MATERIALS AND METHODS

Materials used were the tadpoles of Rhacophorus schlegelii brought up from the eggs collected in Niigata City. Tadpoles were reared on boiled spinach. Solutions constituting experimental environment were changed every 2-4 days. Tadpoles were fixed in Bouin’s solution. Sections were made in 12μ thickness, and stained with Delafield’s hematoxylin and eosin. The acinar cell height of 20 follicles was measured in the thyroids of each animal, and the mean height was calculated. The present study consists of the following two experiments. In the one (Exp. 1), the tadpoles fertilized on May 8 began to receive the treatment on June 10 (average total length: 31 mm). The concentration of the drug employed was 0.002%. The treated animals were killed on September 4. Metamorphosis in controls occurred in the middle ten days of July. While in the other (Exp. 2), the tadpoles fertilized on June 9 were used as materials. The treatment with 0.0125% solution was initiated on July 2 (average total length: 30 mm).
All the treated animals and the half of controls were killed on July 27. Metamorphosis in the remaining controls occurred one or two weeks later.

**RESULTS**

Metamorphic changes of the larvae kept in the 0.0002% solution were scarcely inhibited. While in the 0.002% solution of this substance, metamorphosis was strongly inhibited. It is therefore conceivable that the metamorphosis-inhibiting effect of methylmercaptoimidazole is about ten times or more as strong as that of thiourea. Growth of the treated animals was considerably retarded, especially in the hind limbs. For example, measurement about the fixed materials in Exp. 1 brought about the following results: total length 44.3 ± 3.6, body length 17.5 ± 1.7, hind limb length 4.2 ± 1.4 mm.

![Diagram of thyroids](image)

Fig. 1. Size of the thyroids (ventral view). Camera lucida drawings. a: controls in Exp. 2, b: treated with 0.0125% of methylmercaptoimidazole in Exp. 2, c: treated with 0.002% of methylmercaptoimidazole in Exp. 1.

Generally speaking, as shown in Figure 1, the thyroid glands hypertrophied remarkably in Exp. 1, although the variation in size was considerably large. Gonadal differentiation was more accelerated in the larvae whose thyroid glands greatly hypertrophied. In Exp. 2, the thyroid glands were slightly smaller than those of controls, and the variation in size was also noticeable. As shown in Table 1, the follicular epitheliums of the thyroid glands of the treated animals were remarkably thicker than those of controls. Histological activity of the gland was parallel with the height of the follicular epithelium. It may be said that the results described above are essentially similar to those of thiourea treatment previously reported (Iwasawa, 1956a, 1957b).

In Exp. 2, as shown in Figure 2, the gonads of the treated animals are slightly smaller in size than those of controls. Histological examination on the gonads also revealed the slight retardation of sex differentiation in the treated animals. In Exp. 1, the gonads of the treated larvae can be classified into the four types

<table>
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<th>Table 1. Epithelial cell height of thyroid follicles (μ)</th>
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<td>Exp. 1</td>
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according to their degrees of development (♂). The gonad is of ovoid form and has a smooth surface. Seminiferous tubules are developing, and mitotic figures are observed in some of the germ cells (Fig. 3) (♂!). The size of the gonad is considerably smaller than that of normal females. Anterior half of the gonad shows almost normal testicular structure, but a few degenerating auxocytes are mainly observed in the posterior part of the gonad. It appears that sex reversal of female to male is progressing (Figs. 4 and 5) (♂♀). External view is the same as that of the normal ovary, but histologically, variation in the size of auxocytes is noticeable, and testicular tissue and degenerating oocytes are observed. It seems that sex reversal of female to male has just begun to take place (Fig. 6) (♀). Normal ovary (Fig. 7). Numerical distribution of the larvae classified according to the said types of gonad is shown in Table 2. During the course of the experiment eleven tadpoles died of disease or from accidental causes. These tadpoles were six males and five females. Consequently it is clear that sex reversal of female to male is induced by the treatment with methylmercaptoimidazole.

| Table 2. Distribution of sex in Exp. 1 |
| --- | --- | --- | --- | --- |
| ♂ | ♂♀ | ? | ♀ | Total |
| 14 | 7 | 8 | 1 | 29 |

**COMMENT**

The results obtained by the present study are perfectly compatible with those obtained by thiourea treatment previously reported. It is therefore strongly sug-
gested that the acceleration of gonadal development and the sex reversal of female to male in higher anuran larvae induced by the treatment with thiourea are the common phenomena produced by any antithyroidal thiourea derivatives. Asayama (1955) suggested in virtue of his studies concerning the effects of ICSH or ACTH upon the sex differentiation in Hynobius nebulosus that the hypophysis might participate in the mechanism of sex differentiation of the proteinic and the sterolic endocrine systems, but not by the unitary action of the sterolic endocrine substances. Asayama and Miyamori (1957) further suggested from their experiment with thiourea that both the thyroid gland and the hypophysis might participate in the mechanism of the sex differentiation of embryonic gonad. Hanaoka (1954) supposed that the mechanism of sex reversal induced by the treatment with thiourea might be concerned with the general metabolic changes produced by hormonal disorder. It was described in the previous paper (Iwasawa, 1955a) that the effects of thiourea on the amphibian larvae would include not only the inhibition of thyroid hormone production with consequent over-secretion of thyroid stimulating hormone but also unusual changes of levels of secretion of other hypophyseal hormones together with the unphysiological alteration in the activities of endocrine organs which were under the control of hypophyseal hormones. It is well known that sex differentiation of larval gonad in amphibia is greatly influenced by the application of various steroidal hormones, and there has been considerable discussion concerning the discrimination between these steroids and Witschi's so-called sex inductive substances. But very few workers seem to have undertaken the study on the role of the hypophysis on the sex differentiation. From the author's own observations, it is suggested that the secretory function of the hypophysis begins at an earlier tadpole stage, and the closer relationship between the hypophysis and its subordinate endocrine organs gradually comes into being with the advance of time. Therefore it seems that the study on the influence of hypophyseal hormones upon sex differentiation is, at least in the present state of things, of deep significance. Previously the author reported on the sex reversal induced by the treatment with thiourea which concerns with the functional change of the hypophysis (Iwasawa, 1957c, 1958b), on the abnormal gonadal development produced by the application of p-hydroxypropiophenone (Iwasawa, 1956b, 1958c), and on the abnormal differentiation of larval gonad induced by the injection of hypophyseal gonadotrophin (Iwasawa, 1958d). It is not clear, however, in normal developmental process—not under the unphysiological conditions—whether the revelation of the direction of sex differentiation is concerned with the function of the anterior lobe of the hypophysis, although the development of sex differentiated gonad is greatly influenced by the hypophyseal hormones.

SUMMARY

1. The tadpoles of Rhacophorus schlegelli were treated with methylmercapto-imidazole, and the effects of this substance on the thyroid gland and the gonad were studied.

2. The metamorphosis-inhibiting effect of this substance is about ten times or more as strong as that of thiourea.
3. The influence of this substance on the thyroid gland and the gonad is essentially the same as that of thiourea treatment.

4. The acceleration of gonadal development and the sex reversal of female to male in higher anuran larvae induced by the treatment with thiourea are the common phenomena induced by any antithyroidal thiourea derivatives.

5. It is interesting that the direction of sex differentiation of larval gonad is easily affected by the unusual changes of levels of hypophyseal hormones.

REFERENCES